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Short-Term Tests for Health and Ecological Effects

Part 1: Program Overview
Part 2: Directory of Tests

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16. ABSTRACT

This report is the proceedings of an Office of Health and Ecological Effects (OHEE), U.S. Environmental Protection Agency workshop held at the Research Triangle Park, North Carolina, in January of 1978.

The proceedings consists of eight papers. The first paper is the keynote address; the other seven papers overview the work being done in short-term testing for health and ecological effects by the various U.S. Environmental Protection Agency, Office of Health and Ecological Effects Laboratories.

Included with the proceedings in the Directory of Short-Term Tests for Health and Ecological Effects, which is also published separately as EPA-600/1-78-052. The directory, which was compiled as a result of the workshop, provides basic information about the individual short-term tests for health and ecological effects. The test systems are cross-indexed.

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SHORT-TERM TESTS
FOR HEALTH AND ECOLOGICAL EFFECTS
PART I: PROGRAM OVERVIEW

Sponsored by the

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Hosted by the

Genetic Toxicology Program
Biochemistry Branch
Environmental Toxicology Division
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Research Triangle Park, North Carolina 27711

HEALTH EFFECTS RESEARCH LABORATORY
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This report has been reviewed by the Health Effects Research Laboratory, U.S. Environmental Protection Agency, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the U.S. Environmental Protection Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use. Mention of specific contractors does not necessarily indicate either exclusive engagement or recommendation.

FOREWORD

The many benefits of our modern, developing, industrial society are accompanied by certain hazards. Careful assessment of the relative risk of existing and new man-made environmental hazards is necessary for the establishment of sound regulatory policy. These regulations serve to enhance the quality of our environment in order to promote the public health and welfare and the productive capacity of our Nation's population.

The Health Effects Research Laboratory, Research Triangle Park, conducts a coordinated environmental health research program in toxicology, epidemiology, and clinical studies using human volunteer subjects. These studies address problems in air pollution, non-ionizing radiation, environmental carcinogenesis and the toxicology of pesticides as well as other chemical pollutants. The Laboratory participates in the development and revision of air quality criteria documents on pollutants for which national ambient air quality standards exist or are proposed, provides the data for registration of new pesticides or proposed suspension of those already in use, conducts research on hazardous and toxic materials, and is primarily responsible for providing the health basis for non-ionizing radiation standards. Direct support to the regulatory function of the Agency is provided in the form of expert testimony and preparation of affidavits as well as expert advice to the Administrator to assure the adequacy of health care and surveillance of persons having suffered imminent and substantial endangerment of their health.

Historically, the Health Effects Research Laboratory has made a strong commitment to the development and implementation of short-term tests for potential health effects of environmental agents.

This commitment is evidenced by the formation within this laboratory of two new programs; the Genetic Toxicology Program and the Neurotoxicology Program. These programs will require redirection and commitment of new resources to develop techniques that rapidly evaluate pure chemicals and complex environmental samples for possible genotoxic and neurotic hazard.

Research on short-term tests for health and ecological effects is advancing rapidly throughout the Office of Health and Ecological Effects. This document should prove of significant value in maintaining coordination of the research program during this growth period.

F. G. Hueter, Ph.D.
Director
Health Effects Research Laboratory

PREFACE

At the request of Dr. Delbert Barth, Deputy Assistant Administrator for the Office of Health and Ecological Effects (OHEE), the first OHEE Workshop on Short Term Tests for Health and Ecological Effects was held January 18-20, 1978, at the U.S. Environmental Protection Agency (EPA), Health Effects Research Laboratory, Research Triangle Park (HERL-RTP), North Carolina.

The need for such a meeting, especially in the area of genetic toxicology, was suggested by Dr. Alexander Malcolm of the U.S. Environmental Protection Agency at Narrangansett, Rhode Island. The Workshop was planned and coordinated by Dr. Frode Ulvedal and Dr. George Armstrong of OHEE with the Assistance of Dr. Malcolm and the staff of the Biochemistry Branch, HERL-RTP.

The following objectives of the Workshop were established and transmitted with Dr. Barth's letter of invitation to the seven OHEE laboratories and to the National Center for Toxicological Research:

- Produce a directory listing OHEE's screening system efforts with the address and telephone numbers of the key people associated with these efforts;
- Initiate a continuing dialogue among the various investigators as well as visitations among sister laboratories;
- Formulate an agreement for the coordination of the Agency's efforts in health and ecological bioassays; and
- Identify OHEE's needs for future research in this area.

The following Proceedings of the Workshop reflect the presentations given by the representatives of the OHEE laboratories and the National Center for Toxicological Research. These statements describe the research interests and capabilities of the participating laboratories to perform short-term bioassays

PREFACE (continued)

for potential health and ecological effects of environmental agents. It is hoped that additional workshops will continue to improve coordination among the EPA laboratories involved in short-term tests for health and ecological effects.

A major initiative of the Workshop was the preparation of a directory of the short-term tests currently being performed throughout OHEE. This document, the Directory of Short-Term Tests for Health and Ecological Effects, provides information on the tests themselves, the laboratories where they are being performed, and the key individuals involved. It should prove to be an important Agency and interagency reference to this rapidly growing and challenging field of scientific investigation. The Directory also seeks to further the intent of the Workshop, namely to enhance communication, collaboration, understanding, and appreciation of a major component of the U.S. Environmental Protection Agency's research program.

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CONTENTS

Foreword. iii

Preface iv

Acknowledgment. viii

List of Attendees ix

Introductory Speech, *by Delbert Barth, Ph.D.* 1

Overview of Research on Short-Term Predictive Methods at the Environmental Research Laboratory-Duluth, *by James M. McKim, Ph.D.* 5

Overview of Short-Term Testing at the Environmental Research Laboratory-Corvallis, *by William Miller*. 11

Overview of Short-Term Testing at the Environmental Research Laboratory-Narragansett, *by Alexander R. Malcolm, Ph.D.*. 19

Overview of Short-Term Testing at the Environmental Research Laboratory-Gulf Breeze, *by Steven C. Schimmel*. 27

Overview of Short-Term Testing at the Health Effects Research Laboratory-Research Triangle Park, *by Stephen Nesnow, Ph.D.*. . . . 31

Screening Activities at the Health Effects Research Laboratory-Cincinnati, *by Richard J. Bull, Ph.D.*. 43

Overview of Short-Term Bioassays Performed at the National Center for Toxicological Research, *by Daniel A. Casciano, Ph.D.*. . 49

Directory of Short-Term Tests for Health and Ecological Effects 53

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The oral presentations and written overview papers of each speaker, the participation of each attendee, and the editing and final typing by Northrop Services, Inc., are gratefully acknowledged. Dr. Stephen Nesnow was responsible for the technical editing of the Program Overview.

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Introductory Speech

by

Delbert Barth, Deputy Assistant Administrator

Office of Health and Ecological Effects
U.S. Environmental Protection Agency
Washington, District of Columbia

This morning, I hope to be able to set the stage for what you will do over the course of the next day or two. I will give you some of my impressions as to what the most important things are that should transpire. In short, what we have to do is to unify the Office of Health and Ecological Effects (OHEE); once that is accomplished, we will have to unify the Office of Research and Development (ORD), and then the Environmental Protection Agency (EPA). Many different people within EPA are carrying out many different activities that go in many different directions. These activities are not being adequately coordinated. I feel that even in our own office, the Office of Health and Ecological Effects, we are not fully coordinated. That is the principal purpose for this first meeting. Once we are organized, we will be prepared to sponsor meetings of this sort with a much broader attendance; people from other offices will attend, particularly from such programs as the Office of Toxic Substances (OTS) which is obviously interested in the entire subject.

The importance of biological testing within OHEE is twofold. First, these tests may be used as screening devices to set priorities for additional and more indepth studies. Certainly, we are concerned with short-term acute effects. The effects that we are most concerned about, however, are the chronic ones. Ultimately, very expensive and time-consuming tests are necessary to arrive at the chronic effects from long-term exposure to various kinds of environmental pollutants. If we can devise short-term tests capable of predicting where to look first for chronic effects, our program for chronic effects will be greatly helped.

Secondly, these types of tests may be applied to the entire area of risk assessment. When I say "risk assessment", I am talking in the broadest possible terms. We must define what the data needs are and how to interpret the data in order to conduct a meaningful risk assessment. In dealing with batteries of tests, we are on quite infirm ground if we arrive at the risk associated with various environmental pollutants solely on the basis of a single test or even on the basis of a few tests. Perhaps someday we will be able to do so. At present we must define the capabilities and limitations of the various tests, also the quality assurance procedures that will have to be related to these tests. Ultimately, these tests will have to be written up in "cook-book" fashion in the Federal Register; they must be capable of being conducted by industrial groups on the basis of the descriptions found in the Federal Register.

The objectives of this particular meeting are fourfold. First and foremost, we should have an information exchange. We should bring to each other's attention all of the work that is going on in our respective laboratories in this area. After finding out what these efforts are, we should develop mechanisms to coordinate the ongoing work in all of the various laboratories. Then we should develop the research needs that we perceive in this area. And finally, we should develop a joint planning mechanism to accomplish those research needs in the future.

The very foundation for cooperation has to be laid in the planning stage. This cooperation will be very difficult if we each go and plan independently, start a project moving in a certain direction, and then try to make mid-course corrections in order to avoid overlaps with other programs. It would be much better to avoid the necessity for such corrections before commencing any of the studies.

I will outline some topics that you might consider for special emphasis. Some of these I have already alluded to. Certainly, the capabilities and limitations of all of the tests that we are presently working with will have to be established. When I say "capabilities and limitations," I mean in the broad sense. In short, what we need are error terms. How many times are we going to get false positive results with these tests, or how many times are we going to get false negative results? We need a Research and Development (R and D) program to investigate these questions for the various tests. Within ORD and EPA we also need a mechanism for carrying out routine testing. Once we have established the procedures, I personally do not want to see our research laboratories encumbered with large numbers of routine tests. It is entirely possible that this could be done by a contractor; a captive contract laboratory with a project officer on the R and D side would perhaps suffice. In addition, I foresee the need for a rapid turnaround on aquiring results with respect to some pollutant-of-the-day or pollutant-of-the-month that has been identified for concern. Immediately, everybody wants to know what the effects of this pollutant are and quite often we have virtually no data. Obviously, we need some kind of a procedure to obtain results quickly from at least some of the shorter tests and then to indicate whether further tests are needed. Then, someone must decide whether or not to go ahead with further tests, particularly if they become extremely expensive.

In this whole area of testing, a clear statement assigning responsibilities has not been made by the agency as a whole, i.e., by EPA. There are obvious requirements for such a statement. The Office of Pesticide Programs is very much concerned with this matter as is the Office of Air Programs for the Fuel and Fuel Additive Program. And, of course, OTS is involved. ORD has not clearly specified who does what to whom, when and why in this area. And we haven't done it in OHEE. Hopefully, some draft statements will result from this meeting, designating where these responsibilities should lie. We need some kind of a draft statement to at least say that here is what we think OTS should be doing. Here is what we think ORD should be doing. Here's what OHEE should be doing. Here's what Air, Land, and Water Use should be doing. Here is what the Office of Energy, Minerals, and Industry (OEMI) should be doing, etc. You will not be able to reach any final decision on this as people outside of OHEE are involved. But somewhere, someone has to develop a "straw man" that suggests how these responsibilities should be assigned. As it is now, everybody is going off in his own direction; consequently, these efforts lack coordination.

Finally, we will need a five-year R and D plan for this entire problem area of coordination. I don't expect you to do it at this particular meeting, but perhaps you may be able to outline some of the pieces. This plan should set forth the kind of objectives that should be met, the kind of approaches that should be taken to meet these objectives, how time lines and various milestones should be developed over the course of the five years, and how the resources needed to carry out this R and D plan should be assigned.

That is the end product. However, as I said earlier, I do not expect a polished plan to come out of this; you can't do everything in a single meeting. Whatever you can do in the nature of at least outlining the plan would be extremely helpful in order to build future meetings of this sort with a broader attendance. Once we unify OHEE, then as I indicated, we will be able to bring other people in.

OVERVIEW OF RESEARCH ON SHORT-TERM PREDICTIVE METHODS AT THE ENVIRONMENTAL RESEARCH LABORATORY-DULUTH

by

James M. McKim, Ph.D., Chief

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The Environmental Research Laboratory-Duluth (ERL-DUL) has devoted a considerable amount of research effort toward the development of tests involving aquatic animals. These tests seek to determine the subtle toxic effects caused by low levels of environmental contaminants.

The initial efforts involved the development and laboratory use of chronic toxicity tests, exposing a particular species throughout an entire life-cycle and through all stages of development. Initially only fish were utilized, but more recently several invertebrate species were included. The biological endpoints observed and measured during these chronic tests were survival, growth, deformities, reproduction, and residue buildup. These tests provided the best predictive information available on the concentration of a waterborne pollutant necessary to cause detrimental effects to specific animal populations; these tests are considered at this time, the ultimate laboratory test for predicting "no effect" environmental contaminant concentrations for aquatic animals. Further, a five-year field validation study by this laboratory revealed the chronic toxicity test to be a valid useful tool in establishing environmental concentrations of selected chemicals.

However, these life-cycle tests present several problems:

- Time — each test requires 1 to 2 years to complete,
- Cost — $35,000.00 to $70,000.00 per test,
- Few Species — only four to five freshwater species can be utilized successfully in a life-cycle test, and
- High Risk — it becomes difficult to continue an exposure for a year or more without disease or mechanical problems interrupting the test.

Therefore, to eliminate these problems and to develop our capability to quickly and efficiently evaluate a rapidly growing list of chemical pollutants, a number of short-term predictive tests have been developed and are being evaluated by this laboratory's research staff (see Figure 1). All of the predictive short-term tests in Figure 1 relate back to the data base established earlier by chronic toxicity tests. In other words, the short-term predictive tests are designed to predict the results of chronic tests within a much shorter time frame. The large data base available on chronic toxicity allows a rapid evaluation of new test methods with regard to their usefulness in predicting chronic toxicity. The majority of these short-term tests require several weeks to several months to complete and at a fraction of the cost required for life-cycle tests.

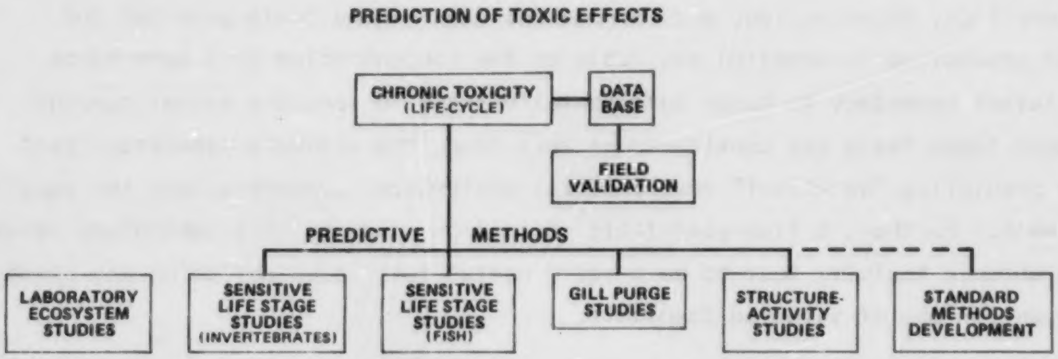
PREDICTION OF TOXIC EFFECTS
CHRONIC TOXICITY (LIFE-CYCLE)
DATA BASE
FIELD VALIDATION
PREDICTIVE METHODS
LABORATORY ECOSYSTEM STUDIES
SENSITIVE LIFE STAGE STUDIES (INVERTEBRATES)
SENSITIVE LIFE STAGE STUDIES (FISH)
GILL PURGE STUDIES
STRUCTURE-ACTIVITY STUDIES
STANDARD METHODS DEVELOPMENT

Figure 1. The short-term predictive toxicity tests being evaluated by ERL-DUL.

The remainder of this overview will contain a series of short summaries on each of the six predictive methods outlined in Figure 1. For more detailed information, please contact the ERL-DUL researcher associated with the summary write-up.

FRESHWATER LABORATORY ECOSYSTEMS FOR TOXICOLOGICAL TESTING (Steven Hedtke)

Traditional aquatic toxicology determines the impact of chemicals on isolated single species. However, in order to evaluate the effects due to the influence of other species, multi-species systems and laboratory ecosystems can be useful. Information on biomagnification, changes in competition and predator-prey interactions, modification of a toxicant's chemical form due to the activity of other species, and changes in ecosystem processes can be obtained. In addition, simultaneously testing several species can be more cost-effective in obtaining fate and effects data.

Specific tests have been utilized or are being developed to monitor changes in periphyton community structure, the uptake of compounds through food/water, and the effect of toxicants on the carbon budget of laboratory ecosystems.

INVERTEBRATE SHORT-TERM TESTS (Richard Anderson, Ph.D.)

Toxicological test systems using invertebrates are as diverse as the animal's habitats. Invertebrate research at ERL-DUL has been directed toward understanding the life cycle of and developing test procedures for cladocerans, amphipods, and aquatic insects. Recent research has centered on the insects. To develop toxicological tests, an understanding of the biology of the test species is required. Two chironomids are now being reared in the laboratory and egg-to-egg test systems are being developed. Tests that expose the animal during specific developmental events are being developed. These tests may decrease the exposure time without decreasing the biological implications of the data. The first series of tests are examining the hatching process. Test systems for mayflies, caddisflies, and stoneflies are also being developed.

TESTS WITH EARLY LIFE STAGES OF FISH FOR PREDICTING LONG-TERM TOXICITY

(James McKim, Ph.D.)

Partial and complete life-cycle toxicity tests with fish, involving all developmental stages, have been used extensively to establish water-quality criteria for aquatic life. During extended chronic exposures of fish to selected toxicants, certain developmental stages have frequently shown a greater sensitivity than others. In 56 life-cycle toxicity tests completed during the last decade with 34 organic and inorganic chemicals and four species of fish, the embryo-larval and early juvenile life stages were the most, or among the most, sensitive. Tests with these stages can be used to estimate the maximum acceptable toxicant concentration (MATC) within a factor or two in most cases. Therefore, toxicity tests with these early life stages of fish should be useful in establishing water-quality criteria and in screening large numbers of chemicals. Work is now under way to further develop both laboratory and field methods for conducting these tests, and to develop new biological endpoints permitting shorter exposure times.

RESPIRATORY IMPAIRMENT IN FISH AS A SHORT-TERM INDICATOR OF TOXICANT STRESS

(Robert Drummond)

Various behavioral and physiological aberrations of fish have been used to indicate stress due to the presence of toxic material. Changes in cough (gill purge) frequency, opercular rate, oxygen consumption, feeding patterns, locomotor activity, fin movements, eye movements, reproductive displays, swimming ability, avoidance, and the ability to respond to a stimulus have all been used as a measure of such stress. Of these, we believe that change in cough frequency has wide potential application as a bioassay tool for the following reasons:

- the response occurs in a variety of fish,
- many chemical substances are known to elicit the response,
- the endpoint is rapid and sensitive,

- the degree of response usually occurs in direct proportion to the toxicant concentration, and
- it is predictive of long-term adverse effects at levels near the MATC values for most of the single toxicants investigated to date.

QUANTITATIVE STRUCTURE-ACTIVITY RELATIONSHIPS (Gilman Veith, Ph.D.)

The application of structure-activity correlations to estimate the toxic effects of organic chemicals to aquatic organisms is being evaluated using a variety of biological test endpoints. A series of phenols are being tested for lethality using 96- and 192-hour acute tests with fathead minnows, 30-day embryo-larval tests, and cough response tests. The effects from the respective tests are correlated with such structural parameters of the chemicals as the n-octanol/water partition coefficient and the Hammet constants. Present research is seeking to determine the precision of toxicity predictions as well as the range of chemicals which can be included in a single correlation.

Additional studies have shown that the chemical residues in fish exposed to a given concentration in water can be estimated using a proportionality factor called the bioconcentration factor. This work has shown a strong correlation between the bioconcentration factor and the n-octanol/water partition coefficient. Current efforts are attempting to develop an instrumental screening technique through this structure — bioconcentration correlation — to minimize the need for costly bioconcentration testing.

Simultaneously, a structure-activity data base is being compiled and appropriate computer software developed to make the data base available on the National Institutes of Health (NIH) and EPA computer systems.

STANDARD METHODS DEVELOPMENT (Charles Stephan)

About 1970 many aquatic toxicologists realized that a need existed both for more cooperation and exhange of information between interested persons as well as for the standardization of terminology and procedures. Today, through the American Society for Testing and Materials (ASTM), interested persons from governmental, industrial, contract, state, and academic organizations are working together on an equal basis to bring about such standardization in aquatic toxicology. The principle concerns at present are procedures for conducting tests, terminology, testing schemes for using various tests, and the culture of test organisms. The results to date show that standardization by consensus of all interested persons does take time and effort, but is probably the most efficient process for producing a product that has wide acceptance.

OVERVIEW OF SHORT-TERM TESTING AT THE ENVIRONMENTAL RESEARCH LABORATORY-CORVALLIS

by

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Special Studies Branch
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and

Jay Gile, Research Biologist
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As a result of the U.S. Environmental Protection Agency's need to establish test procedures and regulatory guidelines for specific inorganic, organic, and complex waste pollutants, the Environmental Research Laboratory-Corvallis (ERL-COR) has established a strong bioassay (screening) program. This research involves both aquatic and terrestrial environments. The screening for ecological effects can and should occur at both the species and community level. Not only should the effects on specific components of an ecosystem be evaluated, but effects on community processes such as nutrient cycling and respiration should also be examined.

AQUATIC RESEARCH

The Aquatic Research Program is classified into freshwater and estuarine-marine categories. The freshwater research centers on the application of the Algal Assay:Bottle Test (AA:BT), the estuarine-marine research on the estuarine-marine version of the AA:BT, the Marine Algal Assay Procedure (MAAP). Both

AA:BT and MAAP define the impact of point and non-point pollutants upon aquatic productivity by measuring algal growth. Estuarine-marine research also includes the Marine Benthos Bioassay.

The AA:BT developed by ERL-COR has been used to evaluate the following three areas:

- nutrient limitation,
- heavy metal toxicity, and
- the inhibitory/stimulatory effects of complex industrial wastes.

Nutrient limitation, defined in natural waters by the AA:BT, may be due to nitrogen, phosphorus, or trace element deficiency. The growth response of Selenastrum capricornutum to single and combined additions of the above-mentioned pollutants is measured by incorporating an internal check and balance system. The responses are evaluated to ascertain the trophic state of the natural water and the limiting nutrients. The AA:BT has proven quite reliable in predicting the algal growth potential of natural water.

The determination of heavy metal toxicity arose out of the evaluation of the nutrient limitation. When a test water, based upon its chemical analysis, fails to attain the predicted yield or nutrient limitation status, the presence of toxicants is usually indicated. The standard addition of 1.0 mg of $Na_2EDTA\ l^{-1}$ to these test waters prior to the assay, is used to define the extent of heavy metal toxicity.

Research conducted at ERL-COR indicated that the inhibition of specific heavy metals upon the growth of Selenastrum capricornutum may be linear (0 to 100%) with increased zinc content in the test waters, but nonlinear for increased copper and cadmium content beyond 20 and 40%, respectively. The growth response of Selenastrum capricornutum to conditions of heavy metal stress in natural waters is in essence a "biological response model" of complex physical and chemical interactions.

Current research activity objectives are to determine if the growth response of the test alga under standard laboratory conditions reflects the response of sensitive indigenous species to recent discharges of heavy metals. These responses may be used to establish realistic heavy metal water quality criteria.

The AA:BT can also be used to define the potential stimulatory/inhibitory effects of complex wastes on algal growth. In one experiment, seven techniques evaluated twenty-three textile waste samples (representing eight manufacturing processes) in order to define their toxic properties. The bioassessment organisms included freshwater and marine algae, crustaceae, fish, and mammals. A comparison of the sensitivity of these bioassays (see Table I) showed that the AA:BT, using Selenastrum capricornutum, was one of the most sensitive tests used in the textile waste survey. This test not only identified toxic wastes; it also identified those that were stimulatory.

The AA:BT protocol has been included in the 14th edition of Standard Methods for the Examination of Water and Wastewater as well as in the EPA Biological Methods Manual. This method is also under review by the American Society of Testing and Materials (ASTM).

When the Toxic Substances Control Act (TSCA) outlined in Public Law 94-469 was established in October of 1976, test procedures and effluent guidelines for pollutants had to be established. A flurry of bioassessment activity was stimulated, as only the bioactive components of the pollutants are responsible for the regulation of biological productivity in natural waters. At the same time, the chemical analysis of specific constituents within the complex wastes (i.e., zinc, copper, cadmium, phenol, PCB, aniline) continued to be accepted as the primary reference standard for the legislation of ecological response criteria; this procedure proved to be both unwise and misleading. Only concurrent evaluation of both chemical analysis and bioassay results can provide the scientific base necessary to establish realistic water quality criteria.

TABLE 1. COMPARATIVE BIOTEST RESPONSES FOR TEXTILE EFFLUENTS[*][†]

	Freshwater ecology series			Recommended interpretation		Marine ecology series		
Textile Plant	Fathead Minnow (96-hr LC50), % secondary effluent	*Daphnia* (48-hr EC50) % secondary effluent	*Selenastrum* (14-day EC50), % secondary effluent	*Selenastrum* 20% secondary effluent % I_{1*}	% S_{1*}	Sheepshead Minnow (96-hr LC50), % secondary effluent	Grass Shrimp (96-hr LC50), % secondary effluent	Algae (96-hr EC50), % secondary effluent
A	19.0	9.0	11.3	53	–	62.0	21.2	f
B	NAT[b]	NAT	–	–	83	NAT	NAT	g
C	46.5	41.0	–	–	187	69.5	12.8	90.0
D	NAT	NAT	–	–	100	f	f	f
E	NAT	7.8	< 2.0	95[e]	–	NAT	NAT	10 to 50
F	NAT	81.7	–	–	598	NAT	NAT	85.0
G	64.7	62.4	–	–	390	NAT	NAT	59.0
H	c	40% dead at 100% concentration	7.8	92	–	f	f	f
J	NAT	NAT	–	–	76	f	f	f
K	NAT	NAT	–	–	57	NAT	NAT	77.0
L	23.5	28.0	12.0	81	–	NAT	NAT	1.7
M	NAT	60.0	–	–	149	f	f	f
N	48.8	100% dead at all dilutions	< 2.0	95[e]	–	47.5	26.3	2.3
P[a]	NAT	NAT	–	–	38	f	f	9.0
R	16.5	8.0	8.8	95	–	f	f	f
S	NAT	NSA[d]	–	–	382	NAT	NAT	g
T	46.5	NAT	–	–	1911	68.0	34.5	70.0
U	NAT	12.1	–	–	377	NAT	NAT	g
V	36.0	9.4	–	–	232	f	f	94.0
W	55.2	6.3	1.0	95	–	37.5	19.6	50.0
X	NAT	NAT	–	–	163	NAT	NAT	g
Y	NAT	NAT	–	–	261	f	f	f
Z	NAT	42.6	15.5	84	–	f	f	f

[a] Sample inadvertently collected prior to settling pond. [b] No acute toxicity. [c] Diseased batch of fish nullified this analysis. [d] No statistical analysis because heavy solids concentration obscured the analysis; the sample does not appear to be acutely toxic. [e] 95% growth inhibition in 2% solution of secondary effluent. [f] Analysis not performed on this sample. [g] Growth inhibition < 50% in 100% solution of secondary effluent. [*] No chemical mutagen was detected by the 10 microbial strains. [†] No rat mortality after 14 days due to maximum dosage of 10^{-3} m^3/kg body weight (LD50). However, six samples (B, C, F, L, N, and S) showed potential body weight effects, and sample R resulted in eye irritation.

The Marine Algal Assay Procedure (MAAP) defines in estuarine-marine waters the aquatic primary productivity of algal growth under the influence of such major nutrients as nitrogen, phosphorus, and silica. This test, using the green flagellate Dunaliella tertiolecta and the green alga Selenastrum capricornutum, can also indicate the nutrient limitation and the potential primary productivity from 0 to 35% salinity under a wide range of nutrient conditions. The MAAP has been included in the 14th edition of Standard Methods for the Examination of Water and Wastewater.

The MAAP has been used to delineate the change from phosphorus-limited freshwater to nitrogen-limited marine water in estuaries, as well as to measure the effect of dredge spoil using the soluble fraction of the Standard Elutriate Test, the toxicity and biostimulation of detergent formulations, and the organochlorine residual in secondary effluents. The test has also monitored the nutrient/physical relationships in Oregon estuaries. Currently, the MAAP is being adapted for use with suspect toxic compounds under the TSCA program.

The Acute Benthos Toxicity Bioassay developed at ERL-COR's Newport Field Station roughly simulates the dumping of dredged materials into the sea. Since the benthos is affected by the settleable fraction of dredged sediments, five macrobenthic invertebrates were selected as the test species. Mean survival compared to the control after 10 days of exposure, is the primary response criterion. Bioassays have been conducted for sediments from the Atlantic, Gulf, and Pacific coasts of the United States. The procedure has been published by the U.S. Corps of Engineers in its Implementation Manual for Ocean Dumping Regulations.

The bioassay also has potential in determining the toxicity of sediment at sewage and dredge material dump sites. An experiment is planned to compare the benthic impact of sediments from the New York Bight sludge dump site and relatively clean areas of the Bight. The results will be correlated with the benthic community structure survey.

TERRESTRIAL RESEARCH

The Terrestrial Research Program at ERL-COR focuses on the development and refinement of short-term tests on single plant species, portions of ecosystems (e.g., soil and litter), and whole simulated ecosystems.

ERL-COR is currently working on two single plant bioassays. One is the Stress Ethylene Bioassay and the other the Acetylene Reduction Test.

The first plant bioassay is based on a plant's normally low production level of hormonal ethylene. However, when plants are subjected to environmental stresses, plant ethylene production increases. During the test, the plants are maintained in a greenhouse in sealed exposure chambers and the pollutant is metered into the chamber.

In a study of ozone stress, for example, the increase in the amount of ethylene produced was influenced by both the amount of ozone applied and the plant species. The stress ethylene test not only appeared to be subject to less variability than visual assessment, but it also seemed more sensitive as evidenced by the lack of visible foliar injury associated with increased ethylene production on pine and potato. The induction of stress ethylene by ozone was a short-lived phenomenon with a duration of up to 48 hours following exposure.

The second plant bioassay relies on the ability of a nodulated plant to reduce acetylene to ethylene. The process is monitored to indicate the activity of the enzyme nitrogenase.

Three types of soil microcosms are in various stages of development at ERL-COR. Research is also being conducted on a whole simulated ecosystem called the Terrestrial Microcosm Chamber.

The first two microcosm assays emphasize the decomposition processess occurring in a soil/litter ecosystem. These systems allow the investigators to examine changes in respiration over time as a result of:

- the variation between "identically" prepared sets of microcosms,
- the soil/litter moisture effects on microcosm respiration,
- the salt quality/quantity effects on microcosm respiration and certain other pollutant effects such as:
    - the effects of microcosm age and time of pollutant addition on microcosm respiration,
    - the effects of high concentrations of various metals and nonmetals on microcosm respiration,
    - the effects on respiration of combinations of two toxic substances, and
    - the effects on respiration of toxic substances on organic matter amended soil/litter microcosms.

The third soil system under development at ERL-COR is an adaption of earlier systems designed at Oak Ridge Laboratory. The underlying rational for this system is that intact excised soil cores most reliably represent the natural system since they retain the soil structure and closely simulate the abiotic and biotic complexity of the ecosystem.

The fourth system used at ERL-COR is the Terrestrial Microcosm Chamber. Within a glass box 1 x 3/4 x 2/3 m high with a plexiglass lid, it has been attempted to simulate a whole ecosystem including sun cycle, temperature ranges, a "rain", "spring", and "groundwater" system, membrane and charcoal-filtered air, a local top soil, biotic components comprising primarily alfalfa and perennial ryegrass, and fauna. The fauna include representatives from soil invertebrates, scavengers, pests, and the gray-tailed vole from the mammals.

The Terrestrial Microcosm Chamber provides information on disposition and metabolism within the system. The following effects can be examined:

- chronic and acute phytotoxicity,
- acute and subacute insecticidal action,
- bioaccumulation,

- fetal transfer of residues,
- altered soil respiration,
- interference with nutrient retention,
- mammilian toxicity, and
- behavioral effects.

Future plans for microcosm research include comparison of the various soil systems with soil processes in the larger microcosms and the incorporation of a soil core system within a larger microcosm for testing such toxic substances as CU^{++}, CH_3HgCl, 2, 4-D, and DDT.

OVERVIEW OF SHORT-TERM TESTING
AT THE ENVIRONMENTAL RESEARCH LABORATORY-NARRAGANSETT

by

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Within the Research Division, primary responsibility for the development and application of screening and other short-term tests lies with the Toxicology Branch. Of the four teams comprising this branch, Marine Toxicology, Response Paramaters, Genetic Toxicology, and Bioaccumulation/Kinetics, the first three are directly involved with short-term endpoints. Several other areas (Mussel Watch, Histopathology, Coastal Environmental Assessment Station [CEAS]), benthic aspects of the response parameters program, and selected assays for bioaccumulation designed primarily for long-term assessments, possess the capacity to provide certain types of short-term, toxicological data. These may be appropriately introduced at a future time. For the purposes of this initial meeting, the overview shall be limited to those systems specifically designed for short-term evaluations.

MARINE TOXICOLOGY TEAM

The Marine Toxicology Team has responsibility for the development of the bioassay techniques that permit the interaction of test organism and pollutant in a manner closely approximating their natural encounter. The program has and shall continue to focus on the development of methodology for a wide variety of estuarine species of both ecological and commercial importance. Species representing different communities, trophic levels, and feeding types are selected so as to permit the evaluation of impact on a wide scale. Although emphasis has been on short-term techniques, efforts have been expanded to include the whole life cycle, utilizing sensitive, early life histories. This permits evaluation and comparison of the short-term and reproductive effects necessary for projections of population viability.

The program is also strongly directed toward problems of bioaccumulation and the significance of body burden to both public health standards and to effects on reproduction and population integrity. Bioassay designs should reflect more closely actual exposure profiles, thus permitting more realistic estimates of impact.

Current research efforts involve the development of whole life-cycle bioassays on molluscs, polychaete annelids, and oppossum shrimp. The life-cycle stages presently being utilized are fertilization, developing embryo, larval, post-larval metamorphosis, juvenile, and adult. Pollutant stress indicators include survival, growth-molting, fecundity, hatchability/embryo viability, feeding rate, respiration, motile behavior, histopathology, cytogenetics, skeletal abnormalities, and biochemical composition. The major assays are summarized in Table I.

Other areas of interest include the design of an exposure system allowing modulation and the simulation of environmental variables such as salinity and temperature. Methodologies developed by the Marine Toxicology Team and other teams of the Toxicology Branch have been incorporated into the Region II Ocean Disposal Permit Testing Program, the EPA Ocean Disposal Manual, and Standard Methods.

RESPONSE PARAMETERS TEAM

A major objective of the Response Parameters Team is to elucidate a series of sublethal response parameters for motile invertebrate larvae and zooplankton. Parameters being studied include patterns and rates of swimming, motile responses to specific stimuli (light, gravity, food, other chemical cue), feeding and energy budget, and histology. To date, indices of larval viability and physiological

TABLE 1. BIOASSESSMENT METHODS FOR HAZARD EVALUATION IN THE MARINE ENVIRONMENT

Organism	Acute	Chronic	Life Cycle	Bioaccum	Contract	Lab	Field	Comments
Phytoplankton	Yes	No	Yes	Possible	Yes	Yes	Yes	Excellent short-term screen; readily available.
Macroalgal	Yes	Yes	No	Yes	No	Yes	Possible	New method; promising but not yet available.
Zooplankton	Yes	Yes	Possible	No	Yes	Yes	No	Sensitive but difficult method; needs work in life-cycle area.
Crustacean Larvae	Yes	Yes	Yes	Yes	Possible	Yes	No	Potentially useful screen; not routinely used.
Grass Shrimp	Yes	Yes	Yes	Yes	Yes	Yes	No	New method; limited information.
Mysids	Yes	Yes	Yes	Yes	Yes	Yes	No	Excellent new technique; moderately sensitive and readily available.
Sheepshead Minnow	Yes	Yes	Yes	Yes	Yes	Yes	No	Excellent for chronic studies; moderate sensitivity.
Embryo/Fry	Yes	Yes	No	Yes	Yes	Yes	No	Excellent measure of sensitive life stage effects.
Molluscan Larvae and Adults	Yes	Yes	Yes	Yes	Yes	Yes	No	Excellent assays for chronic and bioaccumulation; not often contracted; larval stages quite sensitive.
Polychaetes	Yes	Yes	Possible	Yes	Yes	Yes	Possible	New assays not yet widely applied; offer potential for sediment bound toxicants and food chain studies.

condition have been limited primarily to survival, development to post-larval stage, rate of development, stage size, and morphological normality. Preliminary experiments suggest that the selected sublethal parameters proposed above may be sufficiently sensitive to permit the short-term detection of adverse toxicant effects in invertebrate larvae at, or significantly below, levels indicated by general developmental parameters alone.

Present efforts are devoted to exploratory studies to select the principle study species and specific behaviors. The behaviors of choice will be those clearly typical of the field situation and capable of being related to population success. Behavior which can be tested simply is pursued first. Selection of the principal study species for subsequent, indepth investigations is based on:

- duration and season of larvae or zooplankton availability,
- possession of swimming patterns that can be analyzed by the Bugwatcher System (see Figure 1), and
- high sensitivity of organisms to various toxicants (metals, oils, etc.).

Table 2 lists some potential study species and their season of availability.

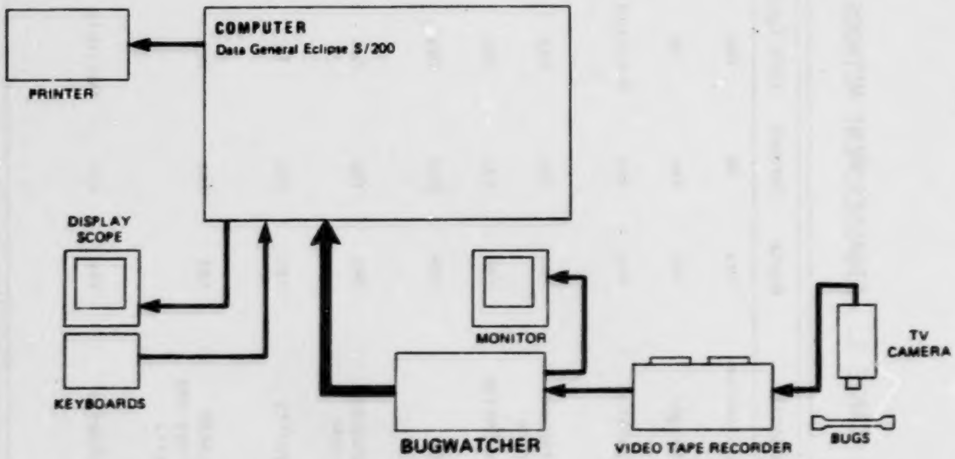

Figure 1. Diagram of the Bugwatcher System.

TABLE 2. POTENTIAL INVERTEBRATE LARVAL AND ZOOPLANKTON STUDY SPECIES

Organism	Season of availability
Crustacean larvae	
Balanus balanoides	March-April
Chthamalus	May-August
B. improvisus	summer -- year round in laboratories
B. amphitrite	summer -- year round in laboratories
Libina	June-September
Cancer	January-August
Crangon	May-October
Palaemonetes	June-September
Zooplankton	
Eurytemora affinus	year round cultures
Mysidopsis bahia	year round cultures
M. bigelowei	year round cultures
Mollusc Larvae	
Crepidula	June-September/October -- year round in laboratories
Nassarium	June-August -- January-August in laboratories
Mulinea	June-August -- January-August in laboratories
Crassostrea	June-August -- January-August in laboratories
Mytilus	June-August -- January-August in laboratories
Rotifera	
Brachionus plicatilis	year round (low-salinity species not of common occurrence in marine systems).

The specific behaviors that are being explored include typical swimming behavior (locomotory rate and patterns) and shifts in locomotory responses to specific environmental stimuli (e.g., light, gravity, temperature, salinity, chemicals-foods, sex pheromone, and alarm substance). All motile behavior studies utilize the computerized behavioral analysis system (Bugwatcher). This system is used to quantify the movement and behavior of small, experimental organisms. With this system, alterations of locomotory or other behavioral patterns of organisms induced by sublethal concentrations of the toxicant can be detected and described. This methodology could represent an early warning system for stress prior to irreversible damage to or death of the animals. The system may also permit rapid screening of suspected toxicants.

GENETIC TOXICOLOGY TEAM

The research conducted by the Genetic Toxicology Team encompasses both mammalian cell and marine assays. At the present time considerable emphasis is being given to the development and application of _in vitro_ mammalian cell assays as indicator systems to identify and assess the DNA-damaging agents in laboratory studies. This program, initially confined to a single assay for gene mutation using nutritional markers, has recently been expanded to include assays for DNA repair and cytogenetic effects. Plans have been formulated to add a transformation test in the near future. In combination, these short-term endpoints span the major types of effects induced by genetic toxicants and represent tests certain to play an increasing role in the regulatory process. Immediate goals include the standardization of each assay with known compounds and the addition of _in vitro_ metabolizing capabilities. The various systems will then be applied to:

- validation,
- improvement of methods for application,
- problem identification, and
- specific research projects to be determined in consultation with other OHEE laboratories.

The mammalian cell assays may also be utilized in conjunction with various monitoring programs such as CEAS and Mussel Watch.

The Gene Mutation Research Program is under the direction of A.R. Malcolm and K.O. Cooper. Current emphasis is on the establishment of an HGPRT locus assay in Chinese Hamster Ovary (CHO) cells and on improvement of the assay employing nutritional markers (CHO cell with 5-bromodeoxyuridine-visible light selection). In the case of the CHO Cell/BrdU-VL system, the major problem is the loss of mutants due to the effects of starvation during selection. Because there are so few markers for measuring mutation in mammalian cells, further development of this system would be justified.

Research on Cytogenetic Effects is being carried out by G.G. Pesch and M.M. Barry. An assay for Sister Chromatid Exchange (SCE) is presently being established with the CHO cell. This cell appears to be well-suited for SCE analysis because of a relatively small chromosome number — 20 — as well as ease of propagation. It is anticipated that this portion of the program will be extended to include some of the more traditional types of chromosomal effects. The assay for SCE has been developed to the point where dose response has been demonstrated with alkylating agents such as ethyl methane sulfonate. Standardization is continuing with mytomycin C and other compounds.

DNA Repair Research is being conducted by E.H. Jackim and W. Lindblad. A test for DNA repair, as measured by unscheduled DNA synthesis, is being implemented with the CHO cell. Although the CHO cell may not be the ultimate cell of choice for the repair test, it has been possible to demonstrate a significant repair response in populations exposed to ultra-violet radiation and certain carcinogenic chemicals. A measurable but less than significant response has been observed with ethyl methane sulfonate. Other standard compounds are being evaluated and the assay is still being refined and modified.

A.R. Malcolm and K.O. Copper will also be responsible for Cell Transformation Research. The transformation test has not yet been initiated, but work with the BALB/c3T3 system is scheduled to begin in June of this year.

The research in Marine Assays is the responsibility of G.G. Pesch and staff. It is well known that coastal environments are often impacted with industrial and domestic discharges containing substantial quantities of metals, organochlorines, oils, etc., many of which may be genetic toxicants. Such toxicants not only accumulate in edible marine organisms where they become a direct threat to man, but may also have a detrimental impact on the genetic structure of natural populations. Initial objectives for the marine portion of the program include:

- the development of short-term assays that can be coupled with long-term, life-cycle assays, and
- the development of methods for _in situ_ monitoring.

The latter should prove particularly valuable for the identification and management of point source problems.

Present research efforts are directed toward the development of methodology for the detection of spontaneous and induced genetic damage in the eggs and larvae of selected marine species under both laboratory and field conditions. Cytogenetic parameters are emphasized. Eggs and larvae comprise the material of choice for technical reasons. These early, rapidly dividing growth stages are particularly sensitive to the action of mutagenic agents and are well-suited to analysis with cytogenetic techniques. An organism of particular monitoring interest is the marine annelid _Neanthes_. This animal is receiving considerable attention as a bioassay tool in several laboratories and has been found to possess a small number — 16 to 18 — of large, metacentric chromosomes.

OVERVIEW OF SHORT-TERM TESTING
AT THE ENVIRONMENTAL RESEARCH LABORATORY-GULF BREEZE

by

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The major mission of the Environmental Research Laboratory-Gulf Breeze (ERL-GB) is to determine the effects of toxic organic chemicals on estuarine biota. The major groups of toxic organics being tested include pesticides, industrial effluents, and carcinogens. ERL-GB is leasing an offshore laboratory 12 miles from land in 110 feet of water. The offshore laboratory provides a capability for toxicity testing in a simulated open ocean environment. Results of studies on these chemicals are published in technical journals, the EPA Research Series, and in the form of written reports to U.S. Environmental Protection Agency Headquarters, Region and State Agencies.

Research at ERL-GB is defined within the framework of two branches: the Experimental Environments Branch, and the Processes and Effects Branch. In addition, a research team has been formed to investigate the impact of carcinogenic materials on estuarine organisms. The Experimental Environments Branch considers the uptake and direct toxic effects of organic chemicals on estuarine plants and animals. The Processes and Effects Branch studies the degradation and fate of toxic organics and the effects of these chemicals on laboratory estuarine ecosystems.

EXPERIMENTAL ENVIRONMENTS BRANCH

Within the Experimental Environments Branch, the complexity of testing ranges from the static, single species bioassay to the multi-species effort of the larval recruitment benthic community studies. The static 96-hour tests provide an estimate of the toxicity of pesticides and industrial wastes to various estuarine crustaceans, molluscs, and fishes. The acute 96-hour toxicity tests provide LC50 data on the same groups of organisms based on measured concentrations of the chemical in seawater. Survivors of these tests are analyzed to estimate the bioconcentration of the chemical. Based on the results of these analyses, the decision may be made to proceed with a long-term (56-day) study to determine the uptake and depuration rates of the chemical and its bioconcentration factor (concentration of the chemical in the animal's tissues divided by the measured concentration of the chemical in the exposure water). In addition to uptake from seawater, studies are conducted to determine the potential of chemicals to be transported within the food web. These studies generally involve primary producers, herbivores, primary and secondary carnivores.

Two crustacean species (Mysidopsis bahia and Palaemonetes pugio) and one fish species (Cyprinodon variegatus) have been developed at ERL-GB for partial or entire life-cycle studies. In these studies, the sensitive early life and reproductive stages are exposed to toxic chemicals in flow-through toxicity tests. Effects measured in these studies are mortality, growth, fecundity, and pathology.

An estuarine community bioassay has been developed at ERL-GB in which unfiltered seawater is passed over a sand substrate, where the larval component of the plankton settle and grow in control or experimental aquaria. Estimates are made for differences in numbers of macrofauna, meiofauna, and biomass. An overall evaluation of shifts in community structure can therefore be determined.

PROCESSES AND EFFECTS BRANCH

The emphasis of the Processes and Effects Branch is on the development of methods to evaluate changes in ecosystem compartments exposed to hazardous inorganic pollutants. The tests under development range from systems that investigate the fate of pollutants in an estuarine environment to systems that focus on how pollutants effect specific behavioral responses. The following systems are being developed:

- Environmental Fate Screening System. The effects of a pollutant are closely related to its availability and chemical form. This system allows investigators to determine the environmental processes and compartments most influential to the movement and transformation of pollutants.
- Eco-Core System. This technique is used to isolate indigenous microorganisms from intact environmental sediment-water cores and to evaluate their degradative potential. The effects of pollutants on microbial ecology are also studied with this system.
- Continuous Flow Systems. These large- and small-scale systems incorporate flowing water and allow the investigation of dynamic environmental processes.
- Aquatic Gradient Avoidance Response System. This system, using behavior as an indicator of chemical exposure, allows quantification of the avoidance responses of estuarine organisms to different pollutant concentration gradients.
- Benthic Bioassay System. Changes in sediment surface features produced by lugworms (*Arenicola cristata*) and exposed to toxicants are used in this system to monitor the effects of pollutants on a benthic infaunal organism.

CARCINOGENESIS RESEARCH TEAM

Energy-related research under the direction of N. Richards, is also being conducted at ERL-GB. Short-term *in vivo* and *in vitro* tests are being developed and validated to detect compounds with mutagenic, teratogenic, and carcinogenic properties from complex environmental mixtures such as shale oil, drilling and packer fluids and cuttings, and biocides. The program consists of the development of methods for the concentration, separation, activation, and detection of toxicants. Concentration and separation techniques are being developed to increase the detection limits of toxicity tests and to separate the interfering substances that are common in tissue residues and other complex environmental mixtures.

The metabolic activation systems being developed include microsomal preparations from a variety of micro-organisms, fish, algae, and crustaceans; these are coupled to the Salmonella typhimurium (Ames) Microsome Test. At the enzyme level, tests are being developed for biphenyl hydroxylase and benzo(a)-pyrene monoxygenase induction. Teratogenic tests include limb regeneration in anthropods, and teratogenic screen tests with isogenic hermaphrodytic fish. Numerous colonies of isogenic inbred fish are being screened in order to develop reference fish for in vivo carcinogen bioassays. Other approaches include:

- the genetic manipulation of fish to produce hybrids with increased carcinogen susceptibility,
- the use of mutant fish selected for carcinogen susceptibility, and
- an evaluation of the use of inbred homozygous, wild type heterozygous, and unisexual diploids that are uniformly heterozygous and triploid unisexuals.

OVERVIEW OF SHORT-TERM TESTING
AT THE
HEALTH EFFECTS RESEARCH LABORATORY-RESEARCH TRIANGLE PARK

Edited by

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The short-term bioassays performed at the Health Effects Research Laboratory-Research Triangle Park (HERL-RTP) constitute an integrated *in vitro* and *in vivo* approach to identifying and assessing hazardous toxicants and genotoxicants present in the environment. Three of the four divisions within the laboratory are involved in in this research: Environmental Toxicology, Experimental Biology, and Clinical Studies Divisions. Their fields of inquiry range from cytotoxicity to carcinogenesis/mutagenesis/teratogenesis to neurobiology and behavior, immunology and host defense. In the ensuing text, the activities conducted by each division are highlighted and summarized according to branch; the names of the investigators who contributed to each summary are listed after the branch title.

ENVIRONMENTAL TOXICOLOGY DIVISION

Biochemistry Branch (Michael Waters, Ph.D., Chief, Biochemistry Branch; Stephen Nesnow, Ph.D., Chief, Metabolic Effects Section; Joellen Huisingh, Ph.D., Chief, Cellular Biology Section)

The short-term bioassay program of the Biochemistry Branch, Environmental Toxicology Division, encompasses three general areas of investigation:

- mutagenesis,
- cellular oncogenic transformation (carcinogenesis), and
- cellular toxicity.

Each area is addressed in an integrated intramural-extramural program that includes basic research, systems development and validation, and systems application components. This program is based on the growing concern for detection of environmental genotoxic agents that may result in an increased incidence of genetic disease and cancer.

The test systems being developed and implemented under the Biochemistry Branch program constitute an integrated battery of methods applicable in screening and mechanistic studies of pure chemicals as well as complex environmental effluents. These bioassay systems are of value both in detecting potentially hazardous materials and in establishing priorities for the testing of suspect substances by conventional (whole animal) toxicological methods.

The application of short-term bioassay systems under the program follows a multi-level approach. The emphasis in Level I testing for mutagens and potential carcinogens and other toxicants is on detection. These systems include gene mutation bioassays in bacteria (e.g., *Salmonella typhimurium*, *Escherichia coli*, *Saccharomyces cerevisciae*) or *Neurospora*. Bioassays for primary DNA damage are included in Level I testing (e.g., *Escherichia coli* pol A^-, *Bacillus subtilis* rec^-). These simple qualitative and quantitative *in vitro* assays for mutagenesis and potential carcinogenesis employ a battery of microbial indicator strains, with an without mammalian microsomal activation systems. The *Salmonella typhimurium* (Ames) Microsome Test, for example, is both an important pre-screening bioassay and an "indicator system", which can be applied to the examination of body fluids and tissues of exposed animals for the presence of mutagens.

An important preliminary component of any battery of short-term tests designed to identify hazardous agents is a search of the literature pertaining to prior testing of chemically related agents. Certain test systems are refractory towards specific chemicals or classes of chemicals due to the nature of the organism or the kind of damage being evaluated. Prior knowledge of the weaknesses of specific bioassays should be incorporated into the decision-making processes for selection of test systems or conditions. It is well known, for example, that chlorinated aliphatic and some aromatic agents are refractory in the *Salmonella typhimurium* (Ames) Microsome Test. In this case, other short-term bioassays would be selected or conventional long-term tests initiated directly.

Level 2 testing emphasizes confirmation of Level I results in appropriate test cells or organisms. The endpoints of mutagenesis and potential carcinogenesis are now separated for greater endpoint definition. Mutagenesis tests in Level 2 involve, for instance, the use of mammalian cells in culture (e.g., L5178Y, V79, CHO cells). Carcinogenesis testing *in vitro* involves morphologic, cellular neoplastic transformation studies (e.g., Syrian hamster embryo cells, C3H10T1/2 or Balb/c3T3 mouse embryo fibroblasts). Level 2 also includes bioassays for chromosomal alterations (*in vitro* and *in vivo* cytogenetics and sister chromatid exchange in mammalian cells, and gene mutation in *Drosophila*, as well as unscheduled DNA synthesis using human cells in culture).

Syrian hamster embryo cells have been used as target cells to assess the carcinogenicity of environmental agents. This neoplastic transformation system has been validated with over 29 known non-carcinogens and 69 known carcinogens. These carcinogens were composed of direct alkylating agents, polycyclic aromatic hydrocarbon, nitrosamines and amides, aromatic amines, azo dyes, and metal salts. There is a 92% concurrence between the *in vitro* results and the *in vivo* data. Of the eight carcinogens which represented the false negatives, seven were subsequently identified as positive when an exogenous source of metabolic activation was added to the bioassay. This gave an overall false negative rate of 1%, a false positive rate of 0%, and an overall concurrence of 99% for the Syrian Hamster Neoplastic Transformation Bioassay.

Level 3 testing, which concludes the toxicological evaluation process, involves the use of conventional whole animal methods. Emphasis is placed on quantitative risk assessment. The *in vitro* methods are valuable in guiding the application of conventional in vivo methodology. In turn, the in vivo procedures provide the required validation of in vivo methods.

All of these bioassay systems are available through a combined intramural-extramural program.

The short-term bioassay program in mutagenesis includes:

- basic mechanistic studies using L5178Y mouse lymphoma cells,
- the development of new assay techniques using Salmonella typhimurium,
- the evaluation of plant mutagenesis assays,
- the evaluation of primary rat hepatocyte DNA repair assays,
- the evaluation of the mutagenicity of diesel exhaust and ambient air samples, and
- the verification, validation, and application of short-term bioassays in batteries of test systems.

In addition, the urine of pesticide treated animals is being examined to evaluate whether or not whole animal metabolism produces mutagenic metabolites.

The development of special techniques for use in the bacterial tester strains for mutagenesis has provided two new environmental monitoring methods:

- the "well test" analyzes extremely small samples with and without mammalian activation systems on a single petri plate;
- the new gas trapping technique using bacteriological methods shows promise for the testing of small ambient air gas samples.

The short-term bioassay program in carcinogenesis centers around the development and use of oncogenic transformation systems (C3H10T1/2CL8 and Balb/c3T3 cells to identify carcinogens, cocarcinogens, tumor initiators, and tumor promoters. The coupling of metabolic activation systems to these systems is currently underway.

The carcinogenesis program has addressed the question as to whether exposure to pesticides increases the carcinogenic or mutagenic potential of environmental genotoxicants. A combination of *in vivo* and short-term bioassay test systems indicate that certain pesticides do increase the mutagenic potential of benzo[a]pyrene, a ubiquitous environmental carcinogen and mutagen. Further studies are in progress to evaluate the effects of pesticides on the carcinogenic potential of benzo[a]pyrene.

A new bioassay using mammalian cells in culture, the Balb/c3T3 Mutagenesis and Oncogenic Transformation Bioassay, has been developed as part of an overall program to develop a battery of short-term test systems that would identify hazardous environmental agents; both the mutagenic and carcinogenic potential of environmental agents are measured. To validate this new system, a number of known carcinogens and mutagens, each representive of a different chemical class, have been used.

The isolated lung perfusion model is assessing the effects of environmental contaminants on the pulmonary metabolism and on the distribution of the environmental carcinogen benzo[a]pyrene. This research is attempting to clarify whether a change in metabolic rate, metabolic pathway, or distribution in the tissues could account for differences in the carcinogenic response.

The short-term bioassay program in toxicity is based on the use of primary cell strains and multiple biochemical endpoints for identifying and categorizing the potency of particulates, environmental effluents, and pure agents. Developmental research has included:

- the evaluation of primary liver cells as carcinogen/mutagen metabolizer cells to be used with existing short-term bioassays;
- the comparison of the carcinogen metabolizing and promutagen activating capabilities of primary liver and lung (alveolar macrophage) cells; and
- the evaluation of the toxicity of a series of organic and inorganic toxic substances using enzymatic and cytological parameters.

In order to effectively integrate the available test systems within the Biochemistry Branch, in vitro and in vivo metabolism must be biochemically well defined. The ability of detection systems to distinguish positives and negatives in chemical screening depends largely upon the extent to which the in vitro metabolism of compounds accurately reflects the in vivo conversions. Knowledge of the ability of cell systems to respond to environmental chemicals by inducing enzymes that activate and detoxify mutagens and carcinogens, is essential in evaluating potential genotoxic effects. For this reason, biochemical indicators that provide information critical to the evaluation of toxicity as well as potential carcinogenicity and mutagenesis, are being developed in conjunction with the in vitro systems described above; they are also being compared to similar indicators in the intact animal.

Toxic Effects Branch (Diane Courtney, Ph.D.)

The Teratology Research Program resides in both the Toxic Effects Branch, Environmental Toxicology Division, and the Developmental Biology Branch, Experimental Biology Division.

Teratology is a beginning research field that needs further exploration. Its short-term testing program is not yet as well developed as in other areas of toxicology. Embryonic and fetal development are much more complex than a single tissue growing in culture. Consequently, the achievement of three-dimensional growth will present some technical problems. The process of solving some of these problems will, however, lead to a broader understanding of embryonic development and maldevelopment.

One approach to short-term teratology testing is a combined in vivo and in vitro procedure in which the pregnant rat is treated from day 6 through 8 of gestation; the embryos are then removed and cultured in vitro for 48 hours. In this approach the embryo is exposed in utero to the toxic agent and any metabolites that might be produced. Advantages of this technique are:

- each embryo serves as its control for development;
- growth can be determined by measuring DNA, protein, and other natural constituents;
- three-dimensional development of the cranial flexures can be observed;
- organ development can be observed by somite counts; and
- observations can be made over time, so that initial lags with subsequent growth can be detected.

This test correlates very well with in vivo testing of teratogenic agents and could become a very valuable testing procedure in teratology.

EXPERIMENTAL BIOLOGY DIVISION

Developmental Biology Branch (Neil Chernoff, Ph.D.)

As mentioned above, the Teratology Research Program is conducted jointly by the Developmental Biology Branch of the Experimental Biology Division and the Toxic Effects Branch of the Experimental Toxicology Division.

Progress in teratogenicity short-term testing has been hampered by a number of factors central to this branch of science. The little that is known about basic mechanisms in teratology has indicated that there is no single trigger for the production of birth defects. One cannot point to a small number of specific preliminary events as predictive of birth defect formation. One problem which short-term teratology testing encounters is the enormous difficulty in duplicating the maternal-fetal relationship between the metabolism of the mother and the placental interface. In vitro or submammalian systems do not allow for these factors and may therefore be impossible to extrapolate. Another reason for the lack of development of short-term testing is that standard teratology testing is considerably shorter than a standard two-year carcinogenicity study, generally taking 3 to 4 months to complete.

One short-term teratogenesis assay that this branch is developing, exposes mouse eggs in vitro to various agents. The four compounds 5-fluorouracil, cadmium, cacodylic acid, and sodium chloride have been examined. Their effect on the following processes was compared:

- rate of cleavage,
- rate of development,
- survival after re-implantation into the dams, and
- uptake of the amino acid leucine.

These tests were not very predictive of tests done in vivo.

Neurobiology Branch (Lawrence Reiter, Ph.D.)

Short-term testing in neurobiology and behavior is aimed at evaluating the effects of environmental pollutants on the functional integrity of the nervous system. In order to thoroughly examine toxicant-induced changes in the central nervous system (CNS) function, scientific investigation must proceed at various levels of neural organization:

- behavioral,
- neurochemical, and
- neurophysiological.

Behavioral analysis provides information on the consequences of toxicant exposure upon the functional output of the nervous system; neurochemical and neurophysiological analysis directly measure nervous system activity.

Within this framework, the Neurotoxicology Program has provided two research functions:

- toxicity testing, and
- methods development and validation.

For toxicity testing, currently existing methods have been utilized to systematically collect information on various neurotoxicants. Methods development and validation, which has proceeded in unison with toxicity testing, focuses on the development of sensitive indices for neurotoxicity that are also simple, rapid, and cost-effective. In the area of behavioral testing, methods range from basic reflexes to naturalistic behaviors to complicated controlled schedules of reinforcement. The short-term behavioral testing procedures evaluate locomotor function, CNS excitability, learning and performance, and social behavior. Neurochemical analyses are primarily concerned with bioenergetics and neurotransmitter function. Finally, the neurophysiological evaluation of CNS function utilizes both electroencephalogram and evoked potential measurements.

CLINICAL STUDIES DIVISION

Biomedical Research Branch (Donald Gardner, Ph.D., Chief)

Short-term tests performed in the Biomedical Research Branch, Clinical Studies Division, use model systems to measure subtle alterations in host defense against airborne toxic substances. Complex cooperative interactions comprising both mechanical and biological components maintain the sterility of the lower airways. Test results indicate that the inhalation of pollutants capable of compromising the functional integrity of any of the individual elements of the pulmonary defense system increase susceptibility to respiratory infection. This increase may be effected by the activation of latent infections once surveillance and defense mechanisms are compromised, or by the failure of host defense systems to respond adequately to subsequent bacterial invasion. Certain populations such as the elderly would be threatened as a result of the decrease in resistance to respiratory infection.

Owing to the problems inherent to the study of the effects of pollutants on the susceptibility of the human respiratory system to microbial infection, animal model systems have been developed to investigate various hypotheses that can later, through appropriate epidemiological surveys, be tested on man. The animal model should reflect a summation of the varied responses of the respiratory tract, including:

- edema,
- cellular disruption,
- reduced macrophage function,
- inflammation, and
- immuno-suppression.

The influence of atmospheric pollutants on the microbe-host interaction is difficult to predict as the test substance may increase, decrease, or have no effect on the susceptibility of the host to infection. The host response is also dependent on the concentration of the pollutant, the physiological state of the host and of the microbial development on and in the host, and the length of time between pollutant exposure and microbial exposure.

A number of different experimental approaches have been used to demonstrate the potential of chemical agents for altering host susceptibility to respiratory infectious agents. One of the most sensitive methods used by a number of laboratories is called the Infectivity Model. Briefly, animals are randomly selected for exposure either to filtered room air or to the test substance. After the cessation of this exposure, the animals from both chambers are combined into a third chamber where they are exposed for approximately 15 min to an aerosol of viable micro-organisms. Micro-organisms that have been employed include: *Streptococcus pyogenes* Group C, *Diplococcus pneumoniae*, *Klebsiella pneumoniae*, *Escherichia coli*, *Salmonella typhimurium*, and influenza virus. At the termination of this exposure some animals from each group are sacrificed and examined, using standard microbiological techniques, to determine the number of inhaled micro-organisms. The remainder of the animals are returned to clean, filtered room air, and the rate of mortality in the two groups is determined during a 15-day holding period. This system can also be expanded to permit observation of altered pathogenic modes, relative mean survival time, and cytological indices of isolated lung cells. Separable elements of the respiratory defense system which can be studied include:

- alveolar macrophage function,
- mucociliary function,
- ciliary beating, and
- mucous physical properties.

Baterial inactivation and killing by alveolar macrophages and rates of clearance can be ascertained by using radiolabeled bacteria. Alveolar macrophages function as primary effectors of host defense against inhaled particulate matter such as bacteria, viruses, and fungi, which are ingested, killed, and degraded by the macrophages. Nonbiological particles such as dusts, soot, etc., are also ingested and degraded to varying extents, unless their chemical composition makes them cytotoxic to macrophages, in which case the particles are released from dead cells to be taken up by other macrophages; the cycle is then repeated. The capacity of a specific pollutant to affect lung macrophage function can also be screened by exposing isolated macrophages in vitro. Specific parameters measured in the macrophage test system include: phagocytosis, viability, cell number, ATP concentration, depression of respiration, and lysosomal enzyme activities.

One of the primary defense systems of the respiratory tract involves mucociliary function. The mucociliary escalator is responsible for the removal of inhaled bacteria and particles deposited in the conducting airways in the respiratory surfaces. The separate mechanisms involved in mucociliary functions are all extremely vulnerable to inhaled pollutants. Ciliary beating has been shown to be inactivated by cigarette smoke, while mucociliary transport is inhibited by a number of substances such as acrolein, formaldehyde, ozone, sulfuric acid, nickel, acetone, cadmium, sulfur dioxide, nitrogen dioxide, ammonia, and cigarette smoke. Assays of ciliastatic activity of various agents are performed in vitro following in vitro or in vivo exposure of the trachea.

In summary, the short-term bioassays performed at the Health Effects Research Laboratory-Research Triangle Park respond to the diverse needs of the U.S. Environmental Protection Agency in the program areas of Pesticides, Toxic Substances, Carcinogenesis, Transportation, Criteria, Non-Criteria, Energy, Environmental Assessment, and Multi-Route. This overall laboratory research effort examines the effects of environmental agents on a multitude of biological species at various levels of biological organization using many biological points. This program focuses on short-term bioassays both as research tools to provide a greater insight into basic mechanisms of toxicity and genotoxicity and as screening tools to aid in the identification of potentially hazardous agents present in our environment.

SCREENING ACTIVITIES AT THE HEALTH EFFECTS RESEARCH LABORATORY-CINCINNATI

by

Richard J. Bull, Ph.D., Chief

Toxicological Assessment Branch
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At the Health Effects Research Laboratory in Cincinnati, Ohio (HERL-CIN), short-term screening activities related to air, water, energy, wastewater reuse, and sludge disposal problems are in progress. The bulk of the effort, however, addresses problems in the water programs.

Screening activities in the water program are required due to:

- a need to acquire epidemiological data indicating relationships between chemical water quality and chronic disease;
- the necessity to identify a wide variety of chemical compounds in finished tap waters:
  - more than 600 organic compounds comprise only 5 to 15% of the total organic carbon;
  - inorganic constituents, including toxic trace metals and asbestos, vary considerably in differing water supplies; and
- the inadequacy of analytical chemistry methods to deal with the major portion of organic material in drinking water, suggesting a need for developing a comprehensive battery of bioassay techniques.

Among those compounds that have been identified are a number of known carcinogens, cardiovascular, hepatic, renal, and hematopoietic toxins. A very large number of the compounds are associated with central nervous system activity; in a few cases the effects have been shown to be irreversible at high doses. Consequently, there is a need for a comprehensive screening program geared to a wide variety of toxicological endpoints.

A fairly comprehensive screening program for damage to deoxyribonculeic acid (DNA) has been in progress for a number of years. The *Salmonella typhimurium* (Ames) Microsome Test is being employed in field studies for correlation with epidemiological data, in the testing of organic concentrates and other environmental samples, as well as individual compounds. This effort, in itself, cannot yield the kind of information required for regulatory decisions. Cell transformation assays are also being explored; however, there is some question as to whether they will add significantly to the solution because of the difficulties involved in their application and the fact that they have many of the same limitations as bacterial systems.

The critical question to be answered in this area is the extent to which the results of any of these tests relate to human disease. The conflict between negative screening test findings with chloroform versus the positive *in vivo* bioassay results from the National Cancer Institute (NCI), also with chloroform, illustrate that these methods cannot be wholly depended upon in circumstances demanding regulatory decisions. For this reason, work that promises to considerably shorten the *in vivo* assessment of damage to genetic material has been emphasized this past year. This seems to be the only reasonable way to take into account the impact of pharmacokinetic, metabolic, and repair factors in order to estimate the degree of risk and modeling dose-response. Information at this level is essential to define the reliability and limitations of simpler test systems. This definition is in turn required for a screening program that can deal reasonably with the problems at hand. The work also incorporates the idea that the methods developed should be applicable to the human for use in prospective epidemiological studies. This final set of information is essential to the validation of simpler test systems. In addition to these efforts, work is being concluded that is specifically designed to determine both the influence of secondary factors such as the immune response in modifying the course of tumorigenesis in experimental animals, and the comparative metabolism of compounds by varying species, including man.

Several epidemiological studies are underway to see if cancer rates may be associated with specific water supplies. Besides both organic and inorganic chemical analyses, the finished drinking waters are being subjected to the Ames test for mutagenesis. On some projects other screening tests for mutagenesis/carcinogenesis are being used. If relationships with cancer rates are found, the screening test may provide an empirical test for the potability of water.

Within the relatively diverse mutagenesis screening programs the need for quality control is obvious. Although the NCI has begun a large quality control program, it appears primarily aimed at standardizing the Ames test. Furthermore, the data from this work will not be available for 1 1/2 to 2 years. There appears to be some advantage in evaluating the diverse systems available to us now, not so much to decide which is the better system, but to get an objective feel for the range of activities that may be detected and the sensitivities of simple bacterial and cell transformation assays. Consequently, HERL-CIN has designed a quality control program based upon putative mechanisms of mutagenic action. It will be offered as an annual service to anyone interested in participating.

Despite this emphasis on mutagenic effects, these are not the effects for which the best epidemiological data exist. The relationship of cardiovascular disease to chemical water quality has been with us since the 1950's. It has been repeated in a number of studies around the world. Estimates are that up to 15% reduction in mortality could result from the identification and elimination of the so-called "water factor." Since the best correlation between water hardness and cardiovascular disease is a negative one, two studies have been set up in whole animals to assess the effects of the major inorganic constituents that change with water hardness. However, if these studies fail to account for the relationship, a severe need for rapid screening techniques will become apparent to deal with the permutations related to the differences in the organic constituencies of hard and soft waters. Although a number of systems may be applicable to this problem, they have not yet been exploited.

A second area that has received little attention beyond gross teratological screening are the effects of chemicals on development and aging. This is an acute problem in the nervous system, since its development in the human is substantially postnatal and by definition falls outside the realm of teratogenic effects. Although some view the area of teratogenesis, development, and aging as being related to mutational events, the bulk of the evidence would suggest that mutational events do not constitute the major mechanism involved. HERL-CIN has developed spectral methods that measure the biochemical development of the brain directly in the tissues. We have found that brain development is significantly and substantially delayed in neonatal rats with peak blood lead (Pb) concentrations of 36 mg Pb/dl, both from a biochemical and morphological point of view. These findings corroborate earlier findings of delayed neurophysiological development of the brain in animals treated with Pb, paralleled by delays in behavioral development. Blood Pb concentrations of this order are certainly within the range encountered in human young in this country. If these data apply to man, as some epidemiological data suggest they do, environmental Pb is having a much greater impact than previously appreciated. Such studies are not long-term, at least in terms of biochemical measurements, involving 3 weeks of lactation. They do demand some devotion of manpower that can, however, be decreased if smaller species with shorter lifespans are used. This could also allow for a more immediate assessment of the long-term functional deficits resulting from such delays in development. It is imperative that such systems be developed in a comparative manner because of possible differences in the internal factors involved in regulating tissue differentiation. However, as these factors are much better understood in mechanistic terms in plants and lower animals than in mammals and humans, a more general approach to chemically-induced developmental problems including teratogenesis may result.

A consideration of utmost importance is the development of biological methods that can be applied to unconcentrated drinking water and source water samples. This sensitivity is required because all current concentration methods delete significant fractions of the material present and all others in the process. As mentioned above, the utilization of the Ames test under such circumstances is already being explored. HERL-CIN is currently investigating those aquatic species that have been used to define ecological hazards associated with chemicals in order to broaden the scope of testing that may be applied. Such systems would be used in a monitoring mode to determine water treatment efficiency, safety of shellfish and crops grown with wastewater and sludge application, as well as to develop criteria for the regulation of individual compounds.

The air program at HERL-CIN has screening requirements that to a large extent are similar to those in the water program. However, specialized needs must be recognized that involve the portal of pollutant entry. Consequently, there is more emphasis on screening for effects directly on the lung and on exploring the pharmocokinetics of the inhalation route. The principle short-term, specialized methods being used include a test assessing the susceptibility of respiratory infections to microbial pathogens and *in vivo* induction of lung tumors on strain A mice. Shorter term systems indicated in the water program have also been used in inhalation exposures; others will be employed as they become validated.

OVERVIEW OF SHORT-TERM BIOASSAYS PERFORMED AT THE NATIONAL CENTER FOR TOXICOLOGICAL RESEARCH

by

Daniel A. Casciano, Ph.D., Research Biologist

Department of Health, Education, and Welfare
Food and Drug Administration
National Center for Toxicological Research
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The primary mission of the National Center for Toxicological Research (NCTR) is to conduct research programs designed to increase the understanding of the biological mechanisms of potentially toxic chemical substances found in man's environment. The research programs are directed toward:

- the development of methodologies and test protocols that evaluate the safety of chemical toxicants;
- the development of data that will facilitate the extrapolation of toxicological data from laboratory animals to man;
- the determination of the basic biological processes for chemical toxicants in animal organisms; and
- the study of the biological effects of potentially toxic chemical substances found in man's environment, with emphasis on the determination of adverse health effects resulting from long-term, low-level exposure to chemical toxicants.

In the past 5 years, the majority of the research effort at NCTR has been oriented toward utilizing *in vivo* mammalian models, primarily the mouse, to help understand the dose-response relationship, especially at very low doses. In addition, in-house research include studies of the metabolism of toxic compounds in cell-free systems and in *in vivo* systems. Studies have also been initiated to develop/modify short-term bioassay systems that would complement the *in vivo* and cell-free studies. The short-term bioassays presently employed at NCTR include:

- the *Salmonella typhimurium* (Ames) Microsome Test;
- the Chinese Hamster Ovary (CHO/HGPRT) Mutagenesis Test; and
- the DNA Repair Activity Test (Unscheduled DNA Synthesis).

The *Salmonella typhimurium* (Ames) Microsome Test is basically that described by Ames except for, in some cases, the source of the metabolic activating enzymes, S9 — prepared from uninduced mouse liver. Since the majority of the *in vivo* experimentation at NCTR uses the mouse as a model, S9 serves as the metabolic activating system. This assay system has been used to study the mutagenic activity of a variety of chemical classes including aromatic amines, polycyclic aromatic hydrocarbons, nitrosamines, direct alkylating agents, and others.

The Chinese Hamster Ovary Mutagenesis Test is basically that described by Hsie, et al. The CHO cells are ideally suited for mutagenesis studies because they are genetically well-characterized, exhibit high cloning efficiency, have a stable karyotype, and are capable of growing in a relatively well-defined medium on a glass or plastic substratum or in suspension with a short population-doubling time of 12 to 13 hours. The induced mutants are selected by virture of resistance to the purine analogue 6-thioguanine. This resistance is due to an alteration at the structural gene which confers hypoxanthine-guanine phosphoribosyl transferase activity. This system has been used, coupled with an exogenous metabolic activating system, to detect promutagens/procarcinogens as well as direct acting mutagens/carcinogens. This assay system has been used (mainly by Dr. A. Hsie through an interagency Agreement [IAG]) to study the mutagenic activity of metals, nitrosamines, aromatic amines, polycyclic aromatic hydrocarbons, alkylating agents, and others.

The DNA Repair Activity Test performed at NCTR measures the induced unscheduled DNA synthesis in rodent hepatocytes. Viable hepatocytes are isolated from the livers of rat or mouse by *in situ* collagenase perfusion and placed in primary culture, exposed to a physical or chemical mutagen/carcinogen; DNA repair activity is then measured as induced unscheduled DNA synthesis. Induced DNA repair activity is assessed by measuring the incorporation of ^{3}H-thymidine using direct scintillation counting of acid precipitable material, cesium chloride gradient analysis, and quantitative autoradiography. The hepatocyte culture system is very useful because the cells retain many of the liver's enzymatic capabilities, thereby acting as an endogeneous source of metabolic activating enzymes and as the target cell. The system has been used to study the DNA repair inducing capabilities of several chemicals from a variety of chemical classes including aromatic amines, polycyclic aromatic hydrocarbons, mycotoxins, alkylating agents, and others.

Other mutagenesis activities at NCTR are in vivo, long-term bioassays that include the heritable translocation assay and the assay of gene products (enzymes) found in the somatic tissue of the offspring of the treated population, detecting induced mutations in male mouse germ cells.

SHORT-TERM TESTS
FOR HEALTH AND ECOLOGICAL EFFECTS
PART II: DIRECTORY OF TESTS

Prepared for the

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By the

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CONTENTS

Abbreviations v
Acknowledgment. vi
Note Regarding the Test Systems vii

1000 Short-Term Tests for Health Effects 1

  1100 General Toxicity. 1
    1110 In-Vitro Systems/Acute. 1
    1120 In-Vivo Systems/Acute 21
    1130 In-Vivo Systems/Subacute. 25
    1140 In-Vivo Systems/Inhalation Toxicology 35
    1150 In-Vivo Systems/Neurobehavioral 47

  1200 Genotoxicity. 57
    1210 Mutagenesis 57
    1220 DNA Damage 73
    1230 Carcinogenesis 83
    1240 Marine Applications 91

  1300 Perinatal Toxicology 99

2000 Short-Term Tests for Ecological Effects 107

  2100 Freshwater and Marine Algae 107

  2200 Freshwater Animals and Plants 111
    2210 Fish Toxicity 111
    2220 Invertebrate Toxicity 117
    2230 Plant Toxicity/Residue. 123
    2240 Ecosystem 125

(continued)

CONTENTS (continued)

2300 Marine Animals 127
    2310 Fish Toxicity. 127
    2320 Invertebrate Toxicity. 131
    2330 Ecosystem. 137

2400 Terrestrial Animals and Plants 141
    2410 Plants 141
    2420 Ecosystem. 145

2500 Physical-Chemical/Bio-Accumulation 149

Indices:

I: Test Systems in Numerical Order 154
II: Test Systems in Alphabetical Order. 160
III: Experimental Subject Listing. 168
IV: Contact Personnel in Alphabetical Order 172
V: U.S. EPA Laboratories in Alphabetical Order 175
VI: U.S. EPA Departmental Listing in Alphabetical Order 176
VII: Grant/Contract Laboratories in Alphabetical Order 180
VIII: Status of Development of the Test Systems 184
IX: Endpoints of the General and Perinatal Toxicity Test Systems. 185
X: Endpoint Distribution of the Genotoxicity Test Systems. 190
XI: Endpoint Distribution of the Ecological Test Systems. 191
XII: Application Distribution Table. 192
XIII: Sample Distribution of the General and Perinatal Toxicity Test Systems. 195
XIV: Sample Distribution of the Genotoxicity Test Systems. 199
XV: Sample Distribution of the Ecological Test Systems. 203

ABBREVIATIONS

ERL-COR	Environmental Research Laboratory-Corvallis, Oregon
ERL-DUL	Environmental Research Laboratory-Duluth, Minnesota
ERL-GB	Environmental Research Laboratory-Gulf Breeze, Florida
ERL-NAR	Environmental Research Laboratory-Narrangansett, Rhode Island
HERL-CIN	Health Effects Research Laboratory-Cincinnati, Ohio
HERL-RTP	Health Effects Research Laboratory-Research Triangle Park, North Carolina
NCTR	National Center for Toxicological Research, Jefferson, Arkansas
OAWM	Office of Air and Waste Management
OEMI	Office of Energy, Minerals, and Industry
OHEE	Office of Health and Ecological Effects
OPP	Office of Pesticide Programs
ORD	Office of Research and Development
OTS	Office of Toxic Substances
OWHM	Office of Water and Hazardous Materials
U.S. EPA	United States Environmental Protection Agency

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NOTE REGARDING THE TEST SYSTEMS

The test system information contained in this document was provided by the workshop participants and their colleaques. The science editors, Dr. Shahbeg Sandhu, Dr. Jeffrey Charles, and Dr. James Mckim, combined data where possible, questioned obvious errors and missing information, and insured uniformity to the extent possible in data sheets and indices. No effort was made to restrict inclusion of any test system or related information. Selection and interpretation of terms related to status of development, applications, complexity (simplest to most complex on a scale of 1 to 4), program office support, etc., were the responsibility of the submitters of the information.

CONTENTS: TEST SYSTEMS

1000 Short-Term Tests for Health Effects 1

1100 General Toxicity. 1

1110 In-Vitro Systems/Acute. 1

1111 Integrated Systems: Lung Organ Culture System, Tissue Homogenates, Purified Enzyme Systems . . 2

1112 Integrated System: Tissue Homogenates, Purified Enzyme Systems. 3

1113 Instrumental Methods of Detecting Functional and Metabolic Damage to Target Tissues. 4

1114 Tracheal Mucociliary Transport. 6

1115 Platelet Secretion Measured by A.P Release. . . . 8

1116 Mammalian Platelet and Fibroblast Impairment. . . 9

1117 Lymphocyte Cytotoxicity 10

1118 Impairment of Neutrophil Phagocytosis 11

1119 Human Lung Fibroblasts (WI38) 12

11110 Chinese Hamster Ovary (CHO) Clonal Toxicity . . . 14

11111 Rabbit Alveolar Macrophage (RAM). 16

11112 Rat Hepatocyte (Liver Cell) 18

11113 Chinese Hamster Ovary (CHO) Cytoxicity and Mutagenicity. 19

1120 In-Vivo Systems/Acute 21

1111 INTEGRATED SYSTEM: LUNG ORGAN CULTURE SYSTEM, TISSUE HOMOGENATES, PURIFIED ENZYME SYSTEMS

Biological Activity Detected: Toxicity.

Principle: Enzyme inhibition and/or induction.

Endpoints: Qualitative: Alteration in enzyme activities and/or concentration of metabolites. Quantitative: Degree of alteration in enzyme activities and/or concentration of metabolites.

Strengths: Excellent indicator for pulmonary fibrosis; Very sensitive early indicators.

Weaknesses: Lacks in specificity in some cases; Terminal; Difficult to extrapolate to human situation.

Status of Development: Being implemented.

Describe: Test systems have been fully developed. Data are being collected.

Applications: Multimedia.

Samples: Pure Chemicals: NO_2, SO_2, Hg, Cd, Mn, Zn, Cu. Complex Mixtures: Transportation Related - diesel.

Duration: 5 years ending in 1979.

Cost/sample or chemical: $85.

Interpretation: This system is a very sensitive measure for the degree of alteration relative to pulmonary fibrosis.

Level of Complexity: 2.

OHEE Laboratory Involved: HERL-CIN, Laboratory Studies Division, Functional Pathology Branch, Biochemistry Section.

Persons to Contact: S.D. Lee, U.S. EPA, HERL-CIN, 26 W. St. Clair St., Cincinnati, OH 45268, (FTS 684-7442).

Grant/Contract Laboratory Involved and Principal Investigators: U. of California Medical Center, San Francisco, CA 94132, R.S. Bhatnagar.

Program Office Support: OPP; OEMI.

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1112 INTEGRATED SYSTEM: TISSUE HOMOGENATES, PURIFIED ENZYME SYSTEMS

Biological Activity Detected: Toxicity.
Principal: Enzyme inhibition and/or induction.
Endpoints: Qualitative: Alteration in enzyme activity and/or concentration of metabolites. Quantitative: Degree of alteration in enzyme activity and/or concentration of metabolites.
Strengths: Early indicators; Probably more sensitive than any other method.
Weaknesses: Lacks in specificity in many cases; Terminal; Difficult to extrapolate to human situation.
Status of Development: Validated.
Describe: Data are being accumulated to validate toxic effects of specific pollutants.
Applications: Multimedia.
Samples: Pure Chemicals: O_3, NO_2, Hg, Cd, SO_2. Complex Mixtures: Ambient - O_3 + SO_2.
Duration: 3 years ending June 1978.
Cost/sample or chemical: $75.
Interpretation: This system provides sensitive early indicators for metabolic/cellular injury and recovery.
Level of Complexity: 2.
OHEE Laboratory Involved: HERL-CIN, Laboratory Studies Division, Biochemistry Section.
Persons to Contact: S.D. Lee, U.S. EPA, HERL-CIN, 26 W. St. Clair St., Cincinnati, OH 45268, (FTS 684-7442).
Grant/Contract Laboratory Involved and Principal Investigators: U. of California School of Medicine, Los Angeles, CA 90032, M.G. Mustafa.
Program Office Support: OHEE; OPP.
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1113 INSTRUMENTAL METHODS OF DETECTING FUNCTIONAL AND METABOLIC DAMAGE TO TARGET TISSUES

Biological Activity Detected: Toxicity.

Principle: Increased functional activity of a tissue requires energy. Consequently, if a tissue's functional activity is stimulated, ATP is hydrolyzed to ADP and P_i which in turn stimulates oxidation of substrate and resynthesis of ATP. These metabolic changes may be observed as increases in oxygen consumption, substrate utilization or as metabolic transients induced in the electron carriers directly in tissues, in-vitro. The kinetics of these metabolic responses to stimulation are sensitive to a wide variety of chemical agents with varying mechanisms of action with both in-vitro and in-vivo treatments.

Endpoints: To this point in time the test has only been developed for brain tissue. Responses are measured in response to electrical pulses or elevation in K concentrations. Qualitative: N/A. Quantitative: Transient redox changes in NAD(P)H, fp, cyt a, b, c; Substrate utilization; Oxygen consumption; Lactic acid output; Neurotransmitter release; Amino acid metabolism; Electrical threshold; Frequency response.

Strengths: In-vitro results may be directly confirmed in-vivo with same parameters; Applicable to a wide variety of mechanisms; Applicable to all aerobic tissues; Involves measurement of the kinetics of going from a resting to an excited state rather than the steady state, thereby greatly increasing sensitivity; Applicable to very small tissue samples (2 to 3 mg).

Weaknesses: Does not lend itself to immediate identification of mechanisms unless there is a direct effect on energy metabolism proper.

Status of Development: Validated.

Describe: The test has been validated with a wide variety of inhibitors of energy and membrane active compounds such as ouabain and saxitoxin. In-vitro and in-vivo treatments with lead, methyl mercury, and alkyltin compounds indicate equivalent or more sensitive measures of effect than other parameters which have been applied to these problems.

Applications: Multimedia.

Samples: Pure Chemicals: All classes. Complex Mixtures: Industrial; Energy Related; Transportation Related.

Duration: 2 weeks/compound in-vitro.

Cost: Not precisely established, estimate $1,000 to $2,000/compound.

Interpretation: The test basically assesses potential for neurotoxicity. It indicates the effect of a chemical somewhere between functional activity and the metabolism induced by functional activity. Further studies are required to determine if the effect is on function or metabolism.

Level of Complexity: 2.

OHEE Laboratory Involved: HERL-CIN, Laboratory Studies Division, Toxicological Assessment Branch.

Persons to Contact: R.J. Bull, U.S. EPA, HERL-CIN, 26 W. St. Clair St., Cincinnati, OH 45268, (FTS 684-7213).

1113 INSTRUMENTAL METHODS OF DETECTING FUNCTIONAL AND METABOLIC DAMAGE TO TARGET TISSUES (continued)

Grant/Contract Laboratory Involved and Principal Investigators: In-house.
Program Office Support: OHEE.
References: 1) Bull, R.J., and A.J. Trevor. J. Neurochem., 10:999-1009, 1972. 2) Bull, R.J., and A.J. Trevor. J. Neurochem., 19:1011-1022, 1972. 3) Cummins, J.T., and R.J. Bull. Biochem. Biophys. Acta, 253:29-38, 1971. 4) Bull, R.J., and J.T. Cummins. J. Neurochem., 21:923-937, 1973. 5) Bull, R.J., and S.D. Lutkenhoff. Neuropharmacol., 14:351-359, 1975. 6) Bull, R.J. J. Neurochem., 26:149-156, 1976. 7) Bull, R.J., P.M. Stanaszek, J.J. O'Neill, and S.D. Lutkenhoff. Envir. Hlth. Perspect., 12:89-95, 1975.

1114 TRACHEAL MUCOCILIARY TRANSPORT

Biological Activity Detected: Toxicity.

Principle: Particulates or gases which are deposited on the ciliated epithelium of conducting airways could adversely affect the major functions of this tissue — that of clearance of particulates and effete cells from the lung.

Endpoints: Qualitative: Cytological and histological examination of trachea exposed to air pollutants in-vivo or in-vitro. Quantitative: Measurement of ciliary beating frequency after in-vitro or in-vivo exposure.

Strengths: The in-vitro exposure model permits rapid dose-response studies for ranking of toxicity which can be followed with rapid dose-response studies after in-vivo exposure; The test is quite sensitive; The in-vivo exposure testing dose-response effects permits results to be used for standard setting and regulatory purposes; The in-vitro exposure model requires small amounts of pollutant sample.

Weaknesses: For the inhalation exposure, relatively large amounts of pollutant sample are required.

Status of Development: Validated.

Describe: Both the in-vitro and in-vivo exposure model have been successfully used for Ni, Cd, H_2SO_4, and carbon, and pollutant mixtures. The model has not been used for screening purposes.

Applications: Air.

Samples: Pure Chemicals: Any chemical likely to be deposited on conducting airways. Complex Mixtures: Ambient; Industrial; Energy Related; Transportation Related; Other - any gas, particulate or combination thereof.

Duration: In-vitro exposure: 2 weeks/dose-response of 1 chemical; In-vivo exposure: 4 weeks/dose-response of 1 chemical.

Cost: Approx. $4,000/dose-response of 1 chemical in-vivo; $2,000/dose-response of 1 chemical in-vitro.

Interpretation: Positive result predictive of damage to clearance mechanisms of lung.

Level of Complexity: 3.

OHEE Laboratory Involved: HERL-RTP, Clinical Studies Division, Biomedical Research Branch.

Persons to Contact: J.A. Graham, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2531).

Grant/Contract Laboratory Involved and Principal Investigators: IIT Research Institute, 10 West 35th Street, Chicago, IL 60616, L. Schiff; Northrop Services, Inc., P. O. Box 12313, Research Triangle Park, NC 27709, B. Adkins; Ball State University, Muncie, IN 47306, D. Adalis; U. of North Carolina, Chapel Hill, NC 27514, A. Collier.

Program Office Support: OHEE; OPP; OEMI; OTS.

References: 1)Adalis, D., D.E. Gardner, F.J. Miller, and D.L. Coffin. Toxic Effects of Cadmium on Ciliary Activity Using a Tracheal Ring Model System. Envir. Res., 13:111-120, 1977. 2) Collier, A.M., and J.B. Baseman. Organ Culture Techniques with Mycoplasma. Ann. N.Y. Acad. Sci., 225:277-289, 1973. 3) Donnelly, G.M., H.F.

1114 TRACHEAL MUCOCILIARY TRANSPORT (continued)

McKean, C.S. Heird, and J. Green. Ciliostasis as a Bioassay. Arch. Envir. Hlth., 28:350-355, 1974.

1115 PLATELET SECRETION MEASURED BY ATP RELEASE

Biological Activity Detected: Toxicity; Pharmacologic modulation.

Principle: Platelet function is important in thrombosis, shock, and most inflammatory reactions. Platelet secretion accompanies the more commonly measured aggregation response, and secretion is more easily measured than aggregation.

Endpoints: Qualitative: N/A. Quantitative: ATP release from platelet suspensions is measured by Lucifirin-luciferase assay.

Strengths: Fast; Reproducible; Does not require expensive equipment; Applicable to studies of human blood; Can employ both in-vivo and in-vitro exposures.

Weaknesses: Have not yet been determined.

Status of Development: Developmental.

Describe: Procedures have not been fully developed.

Applications: Air; Water; Food.

Samples: Pure Chemicals: Most classes. Complex Mixtures: Ambient; Industrial; Energy Related; Transportation Related.

Duration: 1 month to develop in-vitro technique; 3 months to develop in-vivo technique; approx. 1 week to implement.

Cost: For development $15,000; to test chemical less than $5,000/chemical.

Interpretation: A positive result is predictive of possible platelet dysfunction.

Level of Complexity: 2.

OHEE Laboratory Involved: HERL-RTP, Clinical Studies Division, Biomedical Research Branch.

Persons to Contact: G. Hatch, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2531).

Grant/Contract Laboratory Involved and Principal Investigators: N/A.

Program Office Support: OHEE; OPP; OEMI; OTS.

References: 1) Charo, O.F., R.D. Feinman, and T.C. Detwiler. J. Clin. Invest., 60:866-873, 1977.

1116 MAMMALIAN PLATELET AND FIBROBLAST IMPAIRMENT

Biological Activity Detected: Toxicity.

Principle: Contaminant is added to platelets in-vitro. The relative amounts of energy metabolism intermediates are measured. C^{14} adenine is used as a precursor.

Endpoints: Qualitative: Ratios of ATP, ADP, and AMP. Quantitative: N/A.

Strengths: Quantitative; Rapid; Capable of direct interpretation.

Weaknesses: Sensitivity.

Status of Development: Developmental.

Describe: Testing with dilutions of pure compounds and extracts of wastewater.

Applications: Water.

Samples: Pure Chemicals: Hydrocarbons. Complex Mixtures: Ambient - rivers; Other - wastewaters.

Duration: 3 years.

Cost/sample or chemical: $125.

Interpretation: Positive result suggests a possible interference of platelet function in-vivo.

Level of Complexity: I.

OHEE Laboratory Involved: HERL-CIN, Field Studies Division, Toxicological Assessment Branch.

Persons to Contact: H. Pahren, U.S. EPA, HERL-CIN, 26 W. St. Clair St., Cincinnati, OH 45268, (FTS 684-7217).

Grant/Contract Laboratory Involved and Principal Investigators: U. of Colorado Medical Center, 4200 E. 9th Ave., Denver, CO 80262, C.C. Solomons.

Program Office Support: OHEE.

References: Not yet available.

1117 LYMPHOCYTE CYTOTOXICITY

Biological Activity Detected: Toxicity.

Principle: Lymphocytes (T cells) have been shown to have cytotoxic effector actions against neoplastic or other cells. The potential exists that pollutants could adversely affect this function, thereby increasing the risk of the host to the development of neoplastic disease.

Endpoints: Qualitative: N/A. Quantitative: Measurements of lymphocyte cytotoxic activity and lectin induced transformation will be made following in-vitro pollutant exposure.

Strengths: The in-vitro model would permit rapid screening for a significant health parameter; Dose-response studies would permit ranking of pollutant effects; Relatively small quantities of pollutant would be required.

Weaknesses: The in-vitro model is not yet validated. Even after validation, results would have to be confirmed with in-vivo exposure studies, possibly of a chronic nature, before the data could be useful for regulation.

Status of Development: Developmental.
Describe: Highly developmental. No pollutants have yet been tested. Model to be completed March, 1979.

Applications: Multimedia.

Samples: Pure Chemicals: Potentially, any chemical. Complex Mixtures: Ambient; Industrial; Energy Related; Transportation Related; Other - any mixture that does not include gas.

Duration: Not yet determined.

Cost: $175,000/model development.

Interpretation: In-vitro model not validated at present.

Level of Complexity: 3.

OHEE Laboratory Involved: HERL-RTP, Clinical Studies Division, Biomedical Research Branch.

Persons to Contact: J.A. Graham, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2531).

Grant/Contract Laboratory Involved and Principal Investigators: Rockefeller U., 1230 York Ave., New York, NY 10021, M. Bowers.

Program Office Support: OHEE; OPP; OEMI; OTS.

References: 1) Kirchner, H., and R.M. Blaese. In Lymphocyte Recognition and Effector Mechanisms. Acad. Press, N.Y., 1974. pp. 357-361. 2) Lightbody, J., and J.C. Rosenberg. In Lymphocyte Recognition and Effector Mechanisms. Acad. Press, N.Y., 1974. pp. 363-367. 3) Perlman, P., and G. Holm. Adv. Immunol., 11: 117, 1969.

Biological Activity Detected: Toxicity.
Principle: Functional and metabolic disturbance of neutrophils are measured after exposure to contaminant.
Endpoints: Qualitative: Percent phagocytosis and percent killing power are measured. Quantitative: N/A.
Strengths: Rapid; Dose-response of procedure has been shown.
Weaknesses: Sensitivity may be a possible problem if low PPB exposure is necessary.
Status of Development: Developmental.
Describe: Testing with dilutions of pure compounds and extracts of wastewater.
Applications: Water.
Samples: Pure Chemicals: Hydrocarbons. Complex Mixtures: Ambient - rivers; Other - wastewaters.
Duration: 3 years.
Cost/sample or chemical: Not yet determined.
Interpretation: Positive result is predictive of possible damage to neutrophils in-vivo.
Level of Complexity: 3.
OHEE Laboratory Involved: HERL-CIN, Field Studies Division, Toxicological Assessment Branch.
Persons to Contact: H. Pahren, U.S. EPA, HERL-CIN, 26 W. St. Clair St., Cincinnati, OH 45268, (FTS 684-7217).
Grant/Contract Laboratory Involved and Principal Investigators: U. of Colorado Medical Center, 4200 E. 9th Ave., Denver, CO 80262, W.L. Weston.
Program Office Support: OHEE.
References: 1) Tan, J.S., et al. A Modified Assay of Neutrophil Function: Use of Lysostrophin to Differentiate Defective Phagocytosis from Impaired Intracellular Killing. J. Lab. Clin. Med., 78:316, 1971.

1119 HUMAN LUNG FIBROBLASTS (WI38)

Biological Activity Detected: Toxicity.

Principle: Toxicants alter biosynthetic processes leading to a reduction in cell growth and division.

Endpoints: Qualitative: Morphology. Quantitative: Cell number and viability; Total cell protein and DNA; Cell adenosine triphosphate; Incorporation of radio-labelled thymidine, uridine, and leucine.

Strengths: Relatively inexpensive; Rapid; Fewer samples required than for conventional whole animal bioassays; One of the best characterized diploid human cells available for toxicity bioassays.

Weaknesses: Not representative of intact animals, providing only preliminary information about the potential health hazards of the test chemicals; May be replaced by other cell strains as supplies dwindle; The system currently can not be coupled with mutagenecity testing unlike other mammalian cell systems.

Status of Development: Being implemented.

Describe: A number of pure compounds have been evaluated.

Applications: Air; Water.

Samples: Pure Chemicals: Inorganics, Organics, Heavy Metals. Complex Mixtures: Industrial; Energy Related - fly ash; Other - AWT effluent, metal-coated fly ash.

Duration: 20 hr.

Cost/sample or chemical: $500 to $1,000.

Interpretation: Alterations in the basic metabolic processes and cellular structure indicate the potential toxicity of the agent.

Level of Complexity: 2.

OHEE Laboratory Involved: HERL-CIN, Field Studies Division, Toxicological Assessment Branch; HERL-RTP, Environmental Toxicology Division, Biochemistry Branch.

Persons to Contact: N.E. Kowal, U.S. EPA, HERL-CIN, 26 W. St. Clair St., Cincinnati, OH 45268, (FTS 684-7477); M.D. Waters, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2693); J.L. Huisingh, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2537).

Grant/Contract Laboratory Involved and Principal Investigators: Gulf South Research Institute, P. O. Box 26518, New Orleans, LA 70186, N. Gruener; Northrop Services, Inc. P.O. Box 12313, Research Triangle Park, NC 27709, N.E. Garrett.

Program Office Support: OHEE; OEMI.

References: 1) Campbell, J.A., H.F. Stack, M.R. Williams, D. Tillery, N. Custer, B.F. Russell, S.W. King, E.B. Siegel, and N.E. Garrett. Cellular Toxicity of Four Liquid Effluent Samples from Textile Mills: Studies on the Rabbit Alveolar Macrophage, WI38 Human Fibroblast and Chinese Hamster Ovary Cell In-Vitro. Contract Report ESG-TR-78-04 to the U.S. Environmental Protection Agency, Northrop Services, Inc., Research Triangle Park, NC. February 1978. 2) Garrett, N.E., J.A. Campbell, J.L. Huisingh, and M.D. Waters. The Use of Short-Term Bioassay Systems in the Evaluation of Environmental Particulates. In: Proceedings of the Symposium

1119 HUMAN LUNG FIBROBLASTS (W138) (continued)

on the Transfer and Utilization of Particulate Control Technology. Denver, CO, July 24, 1978. In press. 3) Waters, M.D., T.O. Vaughan, D.J. Abernathy, H.R. Garland, C.C. Cox, and D.L. Coffin. Toxicity of Platinum (IV) Salts on Cells of Pulmonary Origin. Envir. Hlth. Perspect., 12:45-56, 1975. 4) Waters, M.D., D.R. Abernathy, H.R. Garland, and D.L. Coffin. Toxic Effects of Selected Metallic Salts on Strain W138 Human Lung Fibroblasts. In-Vitro, 10:342, 1974. 5) Waters, M.D., J.L. Huisingh, and N.E. Garrett. The Cellular Toxicity of Complex Environmental Mixtures. In: Proceedings of the Symposium on the Application of Short-Term Bioassays in the Fractionation and Analysis of Complex Environmental Mixtures. Williamsburg, VA, 1978.

11110 CHINESE HAMSTER OVARY (CHO) CLONAL TOXICITY

Biological Activity Detected: Toxicity.

Principle: The toxicity of the samples is evaluated from clonal growth.

Endpoints: Qualitative: N/A. Quantitative: Colony formation.

Strengths: The CHO cell system is well characterized and able to form discrete colonies from single cells; The cell is phagocytically active in culture and sensitive in toxic particulate materials.

Weaknesses: The cell type may not be representative of metabolically active target cells which receive exposure to environmental toxicants.

Status of Development: Being implemented.

Describe: Protocols are established and environmental samples are being evaluated.

Applications: Air; Water.

Samples: Pure Chemicals: H_2SiO_3, Ni_3S_2. Complex Mixtures: Industrial - textile mill effluents; Energy Related - fly ash; Other - particulate materials: silica, fly ash, Dolomite.

Duration: 6 days.

Cost/sample or chemical: $500 to $1,000.

Interpretation: Biochemical changes in CHO cells exposed to the particulates indicate toxicity.

Level of Complexity: 2.

OHEE Laboratory Involved: HERL-RTP, Environmental Toxicology Division, Biochemistry Branch.

Persons to Contact: J.L. Huisingh, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2537).

Grant/Contract Laboratory Involved and Principal Investigators: Northrop Services, Inc., P.O. Box 12313, Research Triangle Park, NC 27709, N.E. Garrett.

Program Office Support: OEMI.

References: 1) Hsie, A.W., et al. Quantitative Mammalian Cell Genetic Toxicology: Study of the Cytotoxicity and Mutagenicity of Seventy Individual Environmental Agents Related to Energy Technologies and Three Subfractions of a Crude Synthetic Oil in the CHO/HGPRT System. In: Proceedings of the Symposium on Short-Term Bioassays in the Fractionation and Analysis of Complex Environmental Mixtures. Williamsburg, VA, 1978. 2) Campbell, J.A., H.F. Stack, M.R. Williams, D. Tillery, N. Custer, B.F. Russell, S.W. King, E.B. Siegel, and N.E. Garrett. Cellular Toxicity of Four Liquid Effluent Samples from Textile Mills: Studies on the Rabbit Alveolar Macrophage, WI38 Human Fibroblast and Chinese Hamster Ovary Cell In-Vitro. Contract Report ESG-TR-78-04 to the U.S. Environmental Progection Agency, Northrop Services, Inc., Research Triangle Park, NC. February, 1978. 3) Garrett, N.E., J.A. Campbell, J.L. Huisingh, and M.D. Waters. The Use of Short-Term Bioassay Systems in the Evaluation of Environmental Particulates. In: Proceedings of the Symposium on the Transfer and Utilization of Particulate Control Technology. Denver, CO, July 24, 1978. In press.

11110 CHINESE HAMSTER OVARY (CHO) CLONAL TOXICITY (continued)

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11111 RABBIT ALVEOLAR MACROPHAGE (RAM)

Biological Activity Detected: Toxicity.

Principle: Toxic agents alter basic metabolic processes and cellular structure of the macrophage.

Endpoints: Qualitative: Morphology. Quantitative: Cell number and viability; Cell adenosine triphosphate; Phagocytic activity; Total cell protein; Hydrolytic enzyme specific activities.

Strengths: The alveolar macrophage plays an important role in the defense of the lung against inhaled particulate materials; This cell type receives direct exposure to environmental toxicants.

Weaknesses: This in-vitro cell system approximates the response which might be observed in the intact animal.

Status of Development: Being implemented.

Describe: The response of the RAM system to a variety of industrial and energy-related particulates has been studied.

Applications: Air; Water.

Samples: Pure Chemicals: Metal chlorides and sulfates, Metallic oxides. Complex Mixtures: Industrial - textile effluents, aluminum refinery, copper smelter; Energy Related - coal gasification and fluidized bed combustion; Other - metal-coated fly ash.

Duration: 20 hr.

Cost/sample or chemical: $500 to $900.

Interpretation: Changes in cellular adenosine triphosphate and viability by dye exclusion indicate potential toxicity of tested substance.

Level of Complexity: 2.

OHEE Laboratory Involved: HERL-RTP, Environmental Toxicology Division, Biochemistry Branch.

Persons to Contact: M.D. Waters, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2693); J.L. Huisingh, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2537).

Grant/Contract Laboratory Involved and Principal Investigators: IIT Research Institute, 10 West 35th Street, Chicago IL 60616, C. Aranyi: Northrop Services, Inc., P.O. Box 12313, Research Triangle Park, NC 27709, N.E. Garrett.

Program Office Support: OHEE; OEMI; OAWM.

References: 1) Huisingh, J.L., J.A. Campbell, and M.D. Waters. Evaluation of Trace Element Interactions Using Cultured Alveolar Macrophages. In: Pulmonary Macrophage and Epithelial Cells. Conf-760972, Sanders, C.L., R.P. Schneider, G.E. Dagle, and H.A. Hagan, eds. ERDA Symposium Series 43, Technical Information Center, Energy Research and Development Administration, 1977. pp. 346-357. 2) Waters, M.D., J.L. Huisingh, and N.E. Garrett. The Cellular Toxicity of Complex Environmental Mixtures. In: Proceedings of the Symposium on the Application of Short-Term Bioassays in the Fractionation and Analysis of Complex Environmental Mixtures. Williamsburg, VA, 1978. 3) Waters, M.D., D.E. Gardner, and D.L. Coffin. Cytotoxic Effects of Vanadium on Rabbit Alveolar Macrophage In-Vitro. Toxicol. Appl. Pharmacol., 28:253-263, 1974. 4) Waters, M.D., T.O. Vaughan, J.A. Campbell, F.J. Miller, and D.L. Coffin. Screening Studies on Metallic Salts Using the Rabbit phage In-Vitro. Toxicol. Appl. Pharmacol., 28:253-263, 1974. 4) Waters, M.D., T.O. Vaughan, J.A. Campbell, F.J. Miller, and D.L. Coffin. Screening Studies on Metallic Salts Using the Rabbit Alveolar Macrophage. In-Vitro, 10:342-343, 1974. 5) Waters, M.D., D.E. Gardner, C. Arnyi, and D.L. Coffin. Metal Toxicity for Rabbit Alveolar Macrophages In-Vitro. Envir. Res., 9:32-47, 1975. 6) Waters, M.D., T.O. Vaughan, D.J. Abernathy, H.R. Garland, C.C. Cox, and D.L. Coffin. Toxicity of Platinum (IV) Salts for Cells of Pulmonary Origin. Envir. Hlth. Perspect., 12:45-56, 1975.

11112 RAT HEPATOCYTE (LIVER CELL)

Biological Activity Detected: Toxicity.
Principle: Toxic agents alter basic metabolic processes and cellular structure and function of the hepatocyte.
Endpoints: Qualitative: Morphology. Quantitative: Cellular viability; Adenosine triphosphate content; Tyrosine aminotransferase activity; Total cell protein.
Strengths: These primary liver parenchyonal cells resemble the adult liver cell in-vivo morphologically and in many of the biochemical parameters evaluated.
Weaknesses: These cells do not divide and must be isolated from a rat prior to each assay. Since there is rat-to-rat variation, cells from several rats should be used to evaluate each chemical.
Status of Development: Developmental; Being implemented.
Describe: New endpoints are being developed; however, the assay is now being implemented with both inorganic and organic chemicals.
Applications: Multimedia.
Samples: Pure Chemicals: Inorganic salts, Organic solvents, Organic solids. Complex Mixtures: N/A.
Duration: 20 hr.
Cost/sample or chemical: $500 to $1,000.
Interpretation: Alterations in the basic metabolic processes and cellular structure and function of the liver cells determine the potential toxicity of the agent.
Level of Complexity: 3.
OHEE Laboratory Involved: HERL-RTP, Environmental Toxicology Division, Biochemistry Branch, Cellular Biology Section.
Persons to Contact: J.L. Huisingh, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2537).
Grant/Contract Laboratory Involved and Principal Investigators: N/A.
Program Office Support: OHEE; OTS.
References: 1) Huisingh, J.L., J.P. Inmon, L.C. King, K. Williams, and M.D. Waters. The Use of Rat Liver Parenchymal Cells in Evaluating Cellular Response to Toxic Metals and Carcinogenic Polycyclic Aromatic Hydrocarbons. In-Vitro, 13:182, 1977.
2) Waters, M.D., and J.L. Huisingh. In-Vitro Testing for Chemical Toxicity: Mammalian Target Cells. In-Vitro, 13:192, 1977.

11113 CHINESE HAMSTER OVARY (CHO) CYTOTOXICITY AND MUTAGENICITY

Biological Activity Detected: Toxicity; Mutagenicity.

Principle: Toxicity is evaluated from effects on clonal growth. Mutation at the hypoxanthine-guanine phosphoribosyl transferase (HGPRT) locus is determined from mutants isolated in 6-thioguanine containing media.

Endpoints: Qualitative: N/A. Quantitative: Colony formation (cytotoxicity); Mutation frequency.

Strengths: Cytotoxicity and mutagenicity may be studied simultaneously.

Weaknesses: The cell type may not be representative of metabolically active cells which receive exposure to environmental toxicants.

Status of Development: Developmental.

Describe: This assay has been shown to be useful in studies of 70 individual environmental agents related to energy technologies and 3 subfractions of a crude synthetic oil.

Applications: Air; Water.

Samples: Pure Chemicals: Polycyclic hydrocarbons, Metallic compounds, Nitrosamines, Quinoline compounds, Physical agents, Alkylating agents, Nitrogen mustards, and Aromatic amines. Complex Mixtures: Energy Related - synthetic fuel.

Duration: 18 days.

Cost/sample or chemical: $500 to $1,000.

Interpretation: Decreasing clonal growth after exposure of CHO cells indicates potential toxicity of test substance. Increasing numbers of mutants with increasing concentration of the test substance indicate the substance is a potential mutagen.

Level of Complexity: 2

OHEE Laboratory Involved: HERL-RTP, Environmental Toxicology Division, Biochemistry Branch, Cellular Biology Section.

Persons to Contact: J.L. Huisingh, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2537).

Grant/Contract Laboratory Involved and Principal Investigators: Northrop Services, Inc., P.O. Box 12313, Research Triangle Park, NC 27709, N.E. Garrett.

Program Office Support: OHEE; OEMI.

References: 1) Hsie, A.W., et al. Quantitative Mammalian Cell Genetic Toxicology: Study of the Cytotoxicity and Mutagenicity of Seventy Individual Environmental Agents Related to Energy Technologies and Three Subfractions of a Crude Synthetic Oil in the CHO/HGPRT System. In: Proceedings of the Symposium on Short-Term Bioassays in the Fractionation and Analysis of Complex Environmental Mixtures. Williamsburg, VA, 1978. 2) O'Neill, J.P., P.A. Brimer, R. Machanoff, G.P. Hirsch, and A.W. Hsie. A Quantitative Assay of Mutation Induction at the Hypoxanthine-Quanine Phosphoribosyl Transferase Locus in Chinese Hamster Ovary Cells: Development and Definition of the System. Mutat. Res., 45:91-101, 1977. 3) O'Neill, J.P., D.B. Couch, R. Machanoff, J.R. San Sebastian, P.A. Brimer, and A.W. Hsie. A Quantitative Assay of Mutation Induction at the Hypoxanthine-Guanine Phosphoribosyl Transferase Locus in Chinese Hamster Ovary Cells (CHO/HGPRT system): Utilization with a Variety of Mutagenic Agents. Mutat. Res., 45:103-109, 1977.

CONTENTS: TEST SYSTEMS

1120 In-Vivo Systems/Acute 21
    1121 LD50. 22
    1122 Whole Animal LD50 — Oral, Dermal. 23
1130 In-Vivo Systems/Subacute. 25

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1121 LD50

Biological Activity Detected: Toxicity.

Principle: Based on preliminary range finding tests, 10 dose-levels of the test compound are selected in the range from 0% to 100% mortality. Each group would consist of 7 animals. The animals receive one dose and then are observed for a period of 14 days. After the 14-day period, a dose-response (cumulative mortality) curve is plotted and the dose level producing 50% mortality is interpolated.

Endpoints: Qualitative: Clinical signs of toxicity. Quantitative: Cumulative mortality.

Strengths: Can determine a dose-response curve for nearly all test compounds and from that establish a maximum tolerated dose level to use in a multiple dose, longer-ranged study.

Weaknesses: Toxicity of vehicle; Solubility; Vehicle-compound synergism and antagonism.

Status of Development: Validated.

Describe: The chemical is administered by route of interest, condition of animals standardized, and observation period specified. Decisions, however, must be made concerning the type of solvent or vehicle that is most appropriate.

Applications: Multimedia.

Samples: Pure Chemicals: Most classes. Complex Mixtures: Drinking water concentrates.

Duration: Variable.

Cost/sample or chemical: $500.

Interpretation: The establishment of a maximum tolerated dose.

Level of Complexity: 1.

OHEE Laboratory Involved: HERL-CIN, Laboratory Studies Division, Toxicological Assessment Branch.

Persons to Contact: R.J. Bull, U.S. EPA, HERL-CIN, 26 W. St. Clair St., Cincinnati, OH 45268, (FTS 684-7213).

Grant/Contract Laboratory Involved and Principal Investigators: N/A.

Program Office Support: OHEE.

References: 1) Loomis, T.A. Essentials of Toxicology, 2nd Ed. Lea and Febiger, Philadelphia, PA, 1974. pp. 17-25.

1122 WHOLE ANIMAL LD50 — ORAL, DERMAL

Biological Activity Detected: Toxicity.

Principle: Administration of test substance to a sufficient number of rats, over a dosage range resulting in 0% to 100% effects.

Endpoints: Qualitative: Clinical effects. Quantitative: Effective dosage to produce effect, LD50 value.

Strengths: Estimates relative toxicity; Economical; Simple to conduct.

Weaknesses: May not apply to all species; May not correspond with data from other laboratories.

Status of Development: Being implemented.

Describe: Tests currently being conducted on limited basis on relevant selected compounds.

Applications: Multimedia.

Samples: Pure Chemicals: All classes. Complex Mixtures: Other - technical grade materials, formulated products.

Duration: 3 months.

Cost: $5,000/chemical for full battery of tests.

Interpretation: A rating of the toxicity of the various chemicals is obtained.

Level of Complexity: I.

OHEE Laboratory Involved: HERL-RTP, Environmental Toxicology Division.

Persons to Contact: R. Linder, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2701).

Grant/Contract Laboratory Involved and Principal Investigators: N/A.

Program Office Support: OPP.

References: 1) Gainer, T.B. Acute Toxicity of Pesticides. Toxicol. Appl. Pharmacol., 1960.

CONTENTS: TEST SYSTEMS

1130 In-Vivo Systems/Subacute. 25

  1131 Integrated System: General Clinical Pathology 26

  1132 Sleep-Time Study. 27

  1133 Metabolism of Chlorinated Hydrocarbons in Subhuman Primates 28

  1134 Metabolic Profiles. 29

  1135 Model Substrate Metabolism. 30

  1136 Xenobiotic Mechanisms 32

  1137 Oxidant Production by Leukocytes and Alveolar Macrophages Measured by Chemiluminescence . . . 33

  1138 Cyclic Nucleotide Concentrations in Leukocytes and Alveolar Macrophages. 34

1140 In-Vivo Systems/Inhalation Toxicology 35

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1131 INTEGRATED SYSTEM: GENERAL CLINICAL PATHOLOGY

Biological Activity Detected: Systemic effects.

Principle: Measurement of serum constituents, proteins, enzyme activities, hematological parameters such as cell counts, cell morphology in peripheral blood and in bone marrow, measurement of special endocrinologic parameters such as thyroid and adrenal functions, pituitary hormones, etc. Diagnosis of malignancies by measurement of tumor marker proteins, serum isoenzyme patterns, etc. Measurement of urinary constituents.

Endpoints: Qualitative: Technique dependent. Quantitative: Technique dependent.

Strengths: High degree of quality control; Great amount of information is available as to the diagnostic implications of abnormal findings.

Weaknesses: Often not capable of signaling asymptomatic preclinical toxic effects; Selection of tests must be done with care and proper planning to ensure maximal effectivity.

Status of Development: Validated.

Describe: This entry encompasses approximately 800 different varieties of tests that may be performed on animals in-vivo or on biological specimens in-vitro. Usually, a battery of tests will be performed examining various organ functions.

Applications: Multimedia.

Samples: Pure Chemicals: N/A. Complex Mixtures:

Other - biological specimens: blood, urine, bone marrow.

Duration: Continuous.

Cost/sample or chemical: Test dependent.

Interpretation: The totality of a battery is aimed at detecting organ-specific toxic effects.

Level of Complexity: 3.

OHEE Laboratory Involved: HERL-CIN, Laboratory Studies Division, Toxicological Assessment Branch, Systemic and Genetic Effects Group; HERL-CIN, Field Studies Division, Toxicological Assessment Branch.

Persons to Contact: R.J. Bull, U.S. EPA, HERL-CIN, 26 W. St. Clair St., Cincinnati, OH 45268, (FTS 684-7213); J.P. Bercz, U.S. EPA, HERL-CIN, 26 W. St. Clair St., Cincinnati, OH 45268, (FTS 684-7480).

Grant/Contract Laboratory Involved and Principal Investigators: N/A.

Program Office Support: OHEE.

References: Theories and practical techniques as compiled in clinical pathology textbooks: 1) Henry and Davidson. Clinical Diagnosis by Laboratory Methods. 2) Tietz. Clinical Chemistry. 3) Wintrobe. Clinical Hematology.

1132 SLEEP-TIME STUDY

Biological Activity Detected: Identifies the biological activity of a compound indicating a potential for interaction with other compounds.

Principle: Chemicals that induce or inhibit MFO will alter the pharmacological effects of drugs metabolized by MFO. Hexabarbital and zoxogolamin are depressant drugs whose properties are well known in this respect. Animals are given a single dose or multiple doses of the test compound at a tolerated but effective level. Two hours after the final dose they are challenged with an anesthetic dose of hexobarbital. A control group receiving no test compound also receives the hexobarbital. The time is measured from the instant the animals lose their "righting reflex" (ability to right themselves when laid flat on their back) to the time they regain it.

Endpoints: Qualitative: Induction or inhibition of liver enzyme activity. Quantitative: Measured sleep-time.

Strengths: Fast and presumptive assay to determine whether a compound will induce or inhibit liver enzymes; Useful in planning more extensive metabolism studies.

Weaknesses: Changes in rates of metabolism must be documented to conclude the effect mediated via MFO.

Status of Development: Validated.
Describe: N/A.

Applications: Multimedia.

Samples: Pure Chemicals: All classes. Complex Mixtures: Industrial; Energy Related.

Duration: Variable depending on projected properties of test compounds.

Cost: Approx. $200/compound.

Interpretation: The results determine the ability of a compound to induce or inhibit enzymatic systems.

Level of Complexity: 2.

OHEE Laboratory Involved: HERL-CIN, Laboratory Studies Division, Toxicological Assessment Branch; HERL-RTP, Clinical Studies Division, Biomedical Research Branch.

Persons to Contact: R.J. Bull, U.S. EPA, HERL-CIN, 26 W. St. Clair St., Cincinnati, OH 45268, (FTS 684-7213); D.E. Gardner, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2531).

Grant/Contract Laboratory Involved and Principal Investigators: N/A.

Program Office Support: OHEE.

References: 1) Conney, A.H., et al. Adaptive Increases in Drug Metabolizing Enzymes Induced by Phenobarbital and Other Drugs. J. Pharmacol. Exp. Ther., 130:1-8, 1960. 2) LaDu, B.N., H.G. Mandel, and E.L. Way. Fundamentals of Drug Metabolism and Drug Disposition. The Williams and Wilkins Co., Baltimore, MD, 1971.

1133 METABOLISM OF CHLORINATED HYDROCARBONS IN SUBHUMAN PRIMATES

Biological Activity Detected: Comparative metabolism.

Principle: In drug metabolism studies, the primate as a model more often represents humans than any other animal model. It is likely that this is the case for environmental contaminants also.

Endpoints: Qualitative: Comparison of metabolites from various animal species. Quantitative: Quantitative analysis of metabolites from various animal species.

Strengths: The primate is most likely to be representative of man in its metabolic activity toward environmental contaminants.

Weaknesses: Expense and difficulty of working with monkeys.

Status of Development: Validated.

Describe: After dosage, specific enzymes are tested for activity. Excreta samples are chemically analyzed for metabolites.

Applications: Multimedia.

Samples: Pure Chemicals: Chlorinated aliphatic hydrocarbons, Chlorinated aromatic hydrocarbons. Complex mixtures: N/A.

Duration: 3 months.

Cost: $15,000 to $18,000.

Interpretation: Comparison of metabolism from various animal systems.

Level of Complexity: 3.

OHEE Laboratory Involved: HERL-CIN, Exposure Evaluation Branch, Organics Metabolism Section.

Persons to Contact: R.D. Lingg, U.S. EPA, HERL-CIN, 26 W. St. Clair St., Cincinnati, OH 45268, (FTS 684-7463).

Grant/Contract Laboratory Involved and Principal Investigators: U. of Cincinnati, Cincinnati, OH 45221, C. Smith, (Commercial 513 872-5700).

Program Office Support: OHEE.

References: 1) Smith, C., R.D. Lingg, and R.G. Tardiff. Comparative Metabolism of Haloethers. Ann. N.Y. Aca. Sci., 298:111, 1977.

Biological Activity Detected: Toxicity; Biotransformation; Comparative metabolism.
Principle: Identification and quantitative analysis of major metabolites are obtained through the use of modern analytical techniques. From these results, a metabolic pathway for a selected compound can be proposed. The potential of a compound for interaction with macromolecules is better understood.
Endpoints: Qualitative: Identification of major metabolites.
Quantitative: Quantitative analysis of major metabolites.
Strengths: Leads to an understanding of the structural differences in compounds which affect their metabolic disposition and toxicity.
Weaknesses: Difficult to extrapolate to the human condition.
Status of Development: Being implemented.
Describe: Most separation and derivatization techniques have been validated using β-Chloroethers and Trichlorobenzenes as model compounds. Additional work needs to be done on processing mass spectral data.
Applications: Multimedia.
Samples: Pure Chemicals: Halogenated aliphatic hydrocarbons, Halogenated aromatic hydrocarbons. Complex Mixtures: N/A
Duration: 3 months.
Cost/sample or chemical: $10,000 to $15,000.
Interpretation: The determination of metabolic pathways leads to an understanding of the differences in the toxication of a selected chemical in various animal species.
Level of Complexity: 2.
OHEE Laboratory Involved: HERL-CIN, Exposure Evaluation Branch, Organics Metabolism Section; HERL-RTP, Environmental Toxicology Division, Biochemistry Branch, Metabolic Effects Section.
Persons to Contact: R.D. Lingg, U.S. EPA, HERL-CIN, 26 W. St. Clair St., Cincinnati, OH 45268, (FTS 684-7463); S. Nesnow, U.S. EPA, HTRL-RTP, Research Triangle Park, NC 27711, (FTS 629-2693).
Grant/Contract Laboratory Involved and Principal Investigators: N/A.
Program Office Support: OHEE.
References: 1) R.D. Lingg, et al. Fate of Bis (2-Chloroethyl) Ether in Rats after Acute Oral Administration. To be presented at the Seventeenth Annual Meeting of the Society of Toxicology, Mar. 12-16, 1978, San Francisco, CA. 2) R.D. Lingg, W. Kaylor, S.M. Pyle, and R.G. Tardiff. Thiodiglycolic Acid: A Major Metabolite of Bis (2-Chloroethyl) Ether. Submitted to Toxicol. and Appl. Pharmacol. Dec., 1977.

1135 MODEL SUBSTRATE METABOLISM

Biological Activity Detected: Toxicity.

Principle: The model substrate assay attempts to determine the effect of environmental chemicals on metabolic pathways in the living animal. This test involves repeated exposure of the experimental animals (6/treatment group) to the toxicants under investigation. After this pretreatment period the animals receive a single oral dose of the model substrate, ^{14}C-lindane. A useful variation in this assay involves the simultaneous administration of lindane with the toxicants being studied.

Endpoints: Qualitative: Altered metabolite profiles serve as a "fingerprint of toxicant exposure" since xenobiotics have characteristic effects on the metabolic pathways of the model substrate. Quantitative: Induced or inhibited metabolism are measured by GLC analysis and liquid scintillation counting of excreted products.

Strengths: Analysis of excreted metabolites provide direct measure of overall metabolism in living animal; Changes in enzyme activity due to disruption of phospholipid membranes, loss of permeability barriers, and loss of local charge effect are avoided; Anomalous enzyme alterations caused by accumulation of substrate and/or metabolites are avoided since normal clearance mechanisms are functional; Economical and practical since comparative activity of multiple pathways are determined on individual animals; Daily changes in the metabolic pathways of animals repeatedly exposed to toxicants can be determined.

Weaknesses: Requires GLC standards of the model substrate metabolites being determined.

Status of Development: Being implemented.

Describe: The model substrate assay has been successfully employed to study interactions between a variety of environmental chemicals, nutritional stress, and the enzyme systems that detoxify or enhance the toxicity of xenobiotics. The correlation between exposure to xenobiotics, whose metabolites produce the same lesion, and their induction of a model substrate metabolite profile with a common element is being investigated.

Applications: Multimedia.

Samples: Pure Chemicals: Ca, Organochlorine pesticides, Herbicides, Insecticides, Fungosides, Drugs, Toxic substances. Complex Mixtures: Not yet tested.

Duration: Test: 2-week exposure; Analysis: 3 weeks.

Cost: $2,700.

Interpretation: It is thought that the generation of unusual alterations in the relative activity of various metabolic pathways of a model substrate can signal toxic interactions.

Level of Complexity: 3.

OHEE Laboratory Involved: HERL-RTP, Environmental Toxicology Division, Biochemistry Branch.

1135 MODEL SUBSTRATE METABOLISM (continued)

Persons to Contact: R.W. Chadwick, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2750); M.F. Copeland, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2678).

Grant/Contract Laboratory Involved and Principal Investigators: N/A.

Program Office Support: OHEE; OPP; OTS.

References: 1) Chadwick, R.W., C.J. Chadwick, J.J. Freal, and C.C. Bryden. Comparative Enzyme Induction and Lindane Metabolism in Rats Pre-treated with Various Organochlorine Pesticides. Xenobiotica, 7:235-246, 1977. 2) Chadwick, R.W., W.S. Simmons, C.C. Bryden, L.T. Chuang, L.M. Key, and C.J. Chadwick. Effect of Dietary Liquid and Dimethyl Sulfoxide on Lindane Metabolism. Toxicol. and Appl. Pharmacol., 39:391-410, 1977. 3) Chadwick, R.W., M.F. Copeland, and C.J. Chadwick. Enhanced Pesticide Metabolism, a Previously Unreported Effect of Dietary Fiber in Mammals. Food and Cosmetics Toxicol., 1978. In press.

1136 XENOBIOTIC MECHANISMS

Biological Activity Detected: Capacity of chemical to act as a synergist or antagonist.

Principle: Groups of 10 animals/sex/dose level are exposed to test compounds for periods of 3 to 7 days, to test the potential of the compound to induce or inhibit liver enzymes. Dose levels used are the maximum tolerated dose (MTD), 1/2 MTD and 1/4 MTD. Following the dose regimen the animals are sacrificed and liver samples are taken for the enzyme assays.

Endpoints: Qualitative: Hexabarital cytP-450 levels, cytPc reductase, O-demethylase, and other microsomal enzyme activities. Quantitative: Hexabarital cytP-450 levels, cytPc reductase, O-demethylase, and other microsomal enzyme activities.

Strengths: Quick assay to determine drugs' potential to cause liver damage; Does not require large numbers of animals.

Weaknesses: No significant weaknesses.

Status of Development: Validated.

Describe: N/A.

Applications: Multimedia.

Samples: Pure Chemicals: Xenobiotics. Complex Mixtures: N/A

Duration: Variable, 1 week to chronic, depending on whether testing is acute or chronic.

Cost/sample or chemical: Variable, depending on test. 1 week at $35,000/manyear equals approximately $700.

Interpretation: This test determines the potential for synergisms and/or antagonisms mediated through altered xenobiotic metabolisms.

Level of Complexity: 2.

OHEE Laboratory Involved: HERL-CIN, Laboratory Studies Division, Toxicological Assessment Branch; HERL-RTP, Environmental Toxicology Division, Biochemistry Branch, Metabolic Effects Section.

Persons to Contact: R.J. Bull, U.S. EPA, HERL-CIN, 26 W. St. Clair St., Cincinnati, OH 45268, (FTS 684-7213); S. Nesnow, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2693).

Grant/Contract Laboratory Involved and Principal Investigators: N/A.

Program Office Support: OHEE.

References: 1) Hayakwa, T. A Simple Radioisotope Assay for Microsomal Aryl Hydroxylase. Anal. Biochem., 51:501-509, 1973. 2) Dallner, G. Studies on the Structural and Enzymatic Organization of Liver Microsomes. Acta Path. Scand., 166:7-41, 1963. 3) Neal, R.A. Studies on the Mechanisms of Detoxification of Cholinergic Phosphorothioates. J. Pharmacol. Exp. Therap., 148:185-192, 1956. 4) Lucier, G.W. Microsomal Rat Liver UDP Glucuronyl Transferase: Effects of Piperonyl Butoxide. Arch. Biochem. and Biophys., 145: 520-530, 1971. 5) Omura, T. The Carbon Monoxide Binding Pigment of Liver Microsomes: I. Evidence for its Hemoprotein Nature. J. Biol. Chem., 239;2370-2378, 1964.

1137 OXIDANT PRODUCTION BY LEUKOCYTES AND ALVEOLAR MACROPHAGES MEASURED BY CHEMILUMINESCENCE

Biological Activity Detected: Toxicity.

Principle: Oxidant production in alveolar macrophage microbicidal activity is due to alveolar macrophage metabolic activation. Oxidants are measured by measuring light produced by oxidation reactions.

Endpoints: Qualitative: N/A. Quantitative: Production of light as measured in an ATP photometer. The amount of light can be measured in response to purified macrophage stimulates and particles.

Strengths: Very reproducible; Fast; Simple; Does not kill cells; Requires very few cells; Applicable to human blood cells; Several types of oxidant can be measured; In-vivo or in-vitro dose-response tests can be made; In-vivo tests are suitable for standard-setting and regulatory purposes.

Weaknesses: Chemiluminescent reactions are susceptible to many quenching effects and competing reactions; Adequate controls are necessary; For in-vivo exposures, relatively large amounts of pollutant sample are required.

Status of Development: Being implemented.

Describe: Effects of O_3, NO_2, and various particles are being determined.

Applications: Multimedia.

Samples: Pure Chemicals: All transparent chemicals which are not readily oxidized. Complex Mixtures: All materials except those which are very opaque.

Duration: In-vitro exposure: 2 weeks/dose-response of 1 chemical; in-vivo exposure: 4 weeks/dose-response of 1 chemical.

Cost: In-vitro exposure: $5,000/chemical; In-vivo exposure: $10,000/chemical.

Interpretation: Defect in oxidant production predicts possible microbicidal defect in the macrophages.

Level of Complexity: 3.

OHEE Laboratory Involved: HERL-RTP, Clinical Studies Division, Biomedical Research Branch.

Persons to Contact: G. Hatch, U.S. EPA, HERL-RTP, Biomedical Research Branch, Research Triangle Park, NC 27711, (FTS 629-2531).

Grant/Contract Laboratory Involved and Principal Investigators: N/A.

Program Office Support: OHEE; OPP; OEMI; OTS.

References: 1) Hatch, G.E., D.E. Gardner, and D.B. Menzel. J. Exp. Med., 147;183-195, 1978. 2) Rosen, H., and S.J. Klebanoff. J. Clin. Invest., 58:50, 1976.

1138 CYCLIC NUCLEOTIDE CONCENTRATIONS IN LEUKOCYTES AND ALVEOLAR MACROPHAGES

Biological Activity Detected: Toxicity; Presumptive oncogenicity; Pharmacologic modulation of promotion.

Principle: Cyclic AMP and Cyclic GMP are intracellular hormones which modulate cellular functions, including cell proliferation, secretion, and movement. The hormones are measured by radioimmunoassay.

Endpoints: Qualitative: N/A. Quantitative: Concentrations of cyclic nucleotide per cell correlate with cellular activity.

Strengths: Cyclic nucleotide concentrations are easily altered by subtle means; Their concentrations are of central importance to cellular activity; Applicable to human blood cells; In-vivo or in-vitro dose-response studies can be done; In-vivo tests would be suitable for standard setting and regulatory purposes.

Weaknesses: Time consuming; Tedious; Requires a lot of cells; Difficult to establish steady basal or control values; In-vivo exposures will require relatively large amounts of pollutant samples.

Status of Development: Being implemented.

Describe: Effects of NO_2, O_3, and NO are being tested for effects on Cyclic AMP and Cyclic GMP.

Applications: Air; Water; Food; Multimedia.

Samples: Pure Chemicals: Most chemicals. Complex Mixtures: Ambient; Industrial; Energy Related; Transportation Related.

Duration: In-vitro exposure: 2 weeks/chemical for dose-response; In-vivo exposure: 4 weeks/chemical for dose-response.

Cost: In-vitro exposure: $5,000/chemical; In-vivo exposure: $10,000/chemical.

Interpretation: Detection of alteration in cellular hormone metabolism which is important to homeostasis.

Level of Complexity: 2.

OHEE Laboratory Involved: HERL-RTP, Clinical Studies Division, Biomedical Research Branch.

Persons to Contact: G. Hatch, U.S. EPA, HERL-RTP, Biomedical Research Branch, Research Triangle Park, NC 27711, (FTS 629-2531).

Grant/Contract Laboratory Involved and Principal Investigators: N/A.

Program Office Support: OHEE; OPP; OEMI; OTS.

References: 1) Hatch, G.E., W.K. Nichols, and H.R. Hill. J. Immunol., 119:450-456, 1977.

CONTENTS: TEST SYSTEMS

1140 In-Vivo Systems/Inhalation Toxicology 35
  1141 Toxicity of Aerosolized Pollutants/Acute and Subacute. 36
  1142 Toxicity of Gaseous and Vapor Phase/Acute and Subacute. 37
  1143 Inhalation LC50 Tests 38
  1144 Deposition and Clearance of Radioactive Materials Following Inhalation Exposure 39
  1145 Integrated System: Pulmonary Function and Pulmonary Metabolism. 40
  1146 Pulmonary Function in Rats. 41
  1147 Pulmonary Function of Unanesthetized Guinea Pigs 42
  1148 Arterial Blood Gas Measurement in Conscious Rats. 43
  1149 Infectivity Model 44
  11410 In-Vivo Alveolar Macrophage Cytotoxicity. 45

1150 In-Vivo Systems/Neurobehavioral 47

1141 TOXICITY OF AEROSOLIZED POLLUTANTS/ACUTE AND SUBACUTE

Biological Activity Detected: Toxicity.

Principle: Exposure begins with a relatively high concentration, which results in 100% mortality in 1 to 4 hours. The concentration is decreased by factors of 10 until no deaths occur. Performed in rats.

Endpoints: Qualitative: N/A. Quantitative: Deaths of animals being exposed.

Strengths: Non-ambiguous positive or negative results.

Weaknesses: For moderately or low toxic substances the determination of a LC50 may be impractical.

Status of Development: Being implemented.

Describe: The equipment required for aerosol generation in the respirable range is being evaluated.

Applications: Air.

Samples: Pure Chemicals: Pesticides. Complex Mixtures: N/A.

Duration: Variable, 1 week to 2 months.

Cost: $10,000 to $20,000/analysis.

Interpretation: A rating of the relative toxicity in mammalian species is obtained.

Level of Complexity: 3.

OHEE Laboratory Involved: HERL-RTP, Environmental Toxicology Division, Toxic Effects Branch, Inhalation Toxicology Group.

Persons to Contact: J. Charles, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2696).

Grant/Contract Laboratory Involved and Principal Investigators: N/A.

Program Office Support: OPP; OTS.

References: 1) Hinners, R.G., J.K. Burkhart, and C.L. Punte. Arch. Envir. Hlth., 16:194-206, 1968.

1142 TOXICITY OF GASEOUS AND VAPOR PHASE/ACUTE AND SUBACUTE

Biological Activity Detected: Toxicity.
Principle: Exposure begins with a relatively high concentration which results in 100% mortality in 1 to 4 hours. The concentrations are decreased by factors of 10 until no deaths occur. Performed primarily in rats. Physiologic and biochemical parameters are measured.
Endpoints: Qualitative: N/A. Quantitative: Death of animals being exposed. Biochemical parameters, enzymes, and substrate levels are assayed for in surviving animals.
Strengths: Non-ambiguous positive or negative results.
Weaknesses: For moderately or low toxic substances the determination of an LC50 may be impractical.
Status of Development: Being implemented.
Describe: 5 chambers, 20 animals/chambers are in operation.
Applications: Air.
Samples: Pure Chemicals: Gases, Vaporizable liquids, Pesticides, Toxic substances in general. Complex Mixtures: N/A.
Duration: Variable, 1 week to 2 months.
Cost: $10,000 to $20,000/analysis.
Interpretation: A rating of the relative toxicity in mammalian species is obtained.
Level of Complexity: 3.
OHEE Laboratory Involved: HERL-RTP, Environmental Toxicology Division, Toxic Effects Branch, Inhalation Toxicology Group.
Persons to Contact: J. Charles, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2696).
Grant/Contract Laboratory Involved and Principal Investigators: N/A.
Program Office Support: OPP; OTS.
References: 1) Drew, R.T., and S. Laskin. Methods of Animal Experimentation, Vol. 4. N.Y. Academic Press. pp. 1-41. 2) Hinners, R.G., J.K. Burkhart, and C.L. Punte. Arch. Envir. Hlth., 16:194-206, 1968.

1143 INHALATION LC50 TESTS

Biological Activity Detected: Toxicity.

Principle: Animals are exposed to different concentrations of the agent, and lethality is determined over a preset period of time.

Endpoints: Qualitative: N/A. Quantitative: Concentration required to cause death in 50% of the exposed animals.

Strengths: Provides initial data on toxicity.

Weaknesses: Cannot be used to determine subtle changes associated with low level exposure.

Status of Development: Validated.

Describe: Most chemicals and complex mixtures can be examined; extremely toxic or carcinogenic samples cannot be examined as HERL-CIN does not have the necessary facilities.

Applications: Multimedia.

Samples: Pure Chemicals: Most chemicals. Complex Mixtures: Ambient, Industrial; Energy Related; Transportation Related; Other.

Duration: 14 days/study.

Cost: $4,000 to $10,000, test dependent.

Interpretation: A rating of the relative toxicity in mammalian species is obtained.

Level of Complexity: 3.

OHEE Laboratory Involved: HERL-CIN, Laboratory Studies Division, Functional Pathology Branch; HERL-RTP, Clinical Studies Division, Biomedical Research Branch.

Persons to Contact: W. Moore, U.S. EPA, HERL-CIN, 26 W. St. Clair St., Cincinnati, OH 45268, (FTS 684-7431); D.E. Gardner, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2531).

Grant/Contract Laboratory Involved and Principal Investigators: N/A.

Program Office Support: ORD.

References: 1) Moore, W., M. Malanchuk, W. Crocker, D. Hysell, A. Cohen, and J.S. Stara. Whole Body Retention in Rats of Different 191Pt Compounds Following Inhalation Exposures. Envir. Hlth. Perspec., 12:35, 1973. 2) Moore, W., J.S. Stara, W. Crocker, M. Malanchuk, and R. Iltis. Comparison of Cadmium Retention in Rats Following Different Routes of Administration. Envir. Res., 6:473, 1973.

1144 DEPOSITION AND CLEARANCE OF RADIOACTIVE MATERIALS FOLLOWING INHALATION EXPOSURE

Biological Activity Detected: Deposition; Translocation clearance.

Principle: Animals are exposed to radioactive aerosol for 15 to 30 minutes; then they are counted at various intervals of time. Tissues are also taken for analysis.

Endpoints: Qualitative: N/A. Quantitative: Data on clearance, translocation, distribution, and excretion as a factor of time.

Strengths: Provides metabolic parameters on the agent under study.

Weaknesses: Agents used are usually radioactive.

Status of Development: Validated.

Describe: N/A.

Applications: Multimedia.

Samples: Pure Chemicals: Radioactive chemicals. Complex Mixtures: N/A.

Duration: Variable, depending on the goals of the study.

Cost/sample: $3,000 to $5,000, depending on cost of compound.

Interpretation: A measure of the residence tissue in the lung and body tissues for the compound is obtained.

Level of Complexity: 3.

OHEE Laboratory Involved: HERL-CIN, Laboratory Studies Division, Functional Pathology Branch; HERL-RTP, Clinical Studies Division, Biomedical Research Branch.

Persons to Contact: W. Moore, U.S. EPA, HERL-CIN, 26 W. St. Clair St., Cincinnati, OH 45268, (FTS 684-7431); D.E. Gardner, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711 (FTS 629-2531).

Grant/Contract Laboratory Involved and Principal Investigators: N/A.

Program Office Support: ORD.

References: 1) Moore, W., D. Hysell, W. Crocker, and J.S. Stara. Biological Fate of ^{103}Pd in Rats Following Different Routes of Exposure. Envir. Res., 8:234, 1974.

1145 INTEGRATED SYSTEM: PULMONARY FUNCTION AND PULMONARY METABOLISM

Biological Activity Detected: Toxicity.

Principle: Structural changes in lung airways and parenchyma can be evaluated in-vivo by use of appropriate tests of pulmonary function. Concommitant metabolic changes can be estimated in-vitro.

Endpoints: Changes can be measured in intact animals. Qualitative and Quantitative: Changes in pulmonary mechanics, spirometry, and diffusion can be evaluated. Metabolic changes of lung tissue can be estimated in-vitro to establish dose-response relationships.

Strengths: Measurements of pulmonary function accomplished by non-invasive methods; In-vivo dose-response effects more applicable for standard-setting and regulatory purposes.

Weaknesses: Expensive equipment; Time consuming; Requires several pairs of hands; Some tests are still being validated; Parenchymal changes (i.e., development of pulmonary fibrosis, pulmonary emphysema or chronic bronchitis) are of great interest in terms of health effects, but they are usually chronic diseases.

Status of Development: Developmental; Being implemented; Validated. Describe: Different tests are in different stages of development. We are presently measuring lung volumes and capacities (TLL, VC, IC, FRC, RV, V_T), breathing frequency, minute ventilation, diffusing capacity for carbon monoxide, nitrogen washout, and quasi-static pressure volume relationships of the lung and chest wall. We are developing methods to measure dynamic compliance and resistance, maximum flow volume relationships, compliance characteristics of excised lungs, and the single breath oxygen test.

Applications: Air.

Samples: Pure Chemicals: Oxides of N and S, Pulmonary toxicants (e.g. Paraquat), Pesticides. Complex Mixtures: Ambient - NO_2, SO_2, O_3; Energy Related - particulates and NO_x, SO_x, O_3, organics, others; Transportation Related - diesel; Other - Toxic substances, Any compound which changes pulmonary physiology.

Duration: 3 month/dose-response of 1 chemical.

Cost: $30,000/dose-response of 1 chemical.

Interpretation: These tests are sensitive and can detect small changes in lung physiology.

Level of Complexity: 3 to 4.

OHEE Laboratory Involved: HERL-RTP, Clinical Studies Division, Biomedical Research Branch.

Persons to Contact: J.J. O'Neil, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2711/2531).

Grant/Contract Laboratory Involved and Principal Investigators: N/A.

Program Office Support: OHEE; OPP; OEMI; OTS.

References: 1) Koo, K.W., et al. Respiratory Mechanics in Normal Hamsters. J. Appl. Physiol., 40:936-942, 1976. 2) Diamond, L., and M. O'Donnell. Pulmonary Mechanics in Normal Rats. J. Appl. Physiol., 43:942-948, 1977. 3) Takezawa, J., F. Miller, and J.J. O'Neil. Single Breath Diffusing Capacity and Lung Volumes in Small Laboratory Animals. J. Appl. Physiol., 1979. In preparation.

1146 PULMONARY FUNCTION IN RATS

Biological Activity Detected: Toxicity.

Principle: Residual volume of the lung increases with many types of obstructive and destructive lung damage. The slope of the static compliance of the pressure-volume curve of the lungs increases with fibrosis and decreases with diseases such as emphysema that destroy alveolar tissue.

Endpoints: Qualitative: N/A. Quantitative: Residual volume (cc) and the slope of the static compliance curves.

Strengths: The measurements are sensitive indicators of lung volume; They are relatively easy to perform.

Weaknesses: The measurements are conducted with rats; Pulmonary anatomy and susceptibility of these animals may differ somewhat from man.

Status of Development: Validated.

Describe: Rats have shown large changes in residual volume and in the slope of the static compliance curve, after 1 to 14 days of exposure to either 0.75 or 1.0 ppm ozone. A manuscript describing these results is being prepared.

Applications: Air; Water; Food.

Samples: Pure Chemicals: Sulfates, Oxidants, Heavy metals. Complex Mixtures: Ambient - air; Industrial - coal dust; Energy Related - stack gases; Transportation Related - diesel and gasoline exhaust; Other - asbestos.

Duration: 1 day to 30 days.

Cost/sample or chemical: $1,000/animal.

Interpretation: An increase in the slope of the static compliance curve and/or an increase in residual volume is indicative of either pulmonary edema or emphysema.

Level of Complexity: 3.

OHEE Laboratory Involved: HERL-CIN, Laboratory Studies Division, Functional Pathology Branch.

Persons to Contact: W.E. Pepelko, U.S. EPA, HERL-CIN, 26 W. St. Clair St., Cincinnati, OH 45268, (FTS 684-7437).

Grant/Contract Laboratory Involved and Principal Investigators: N/A.

Program Office Support: OHEE.

References: 1) J. Appl. Physiol., 26:738-744, 1966. 2) Chest, 475-481, 1967.

1147 PULMONARY FUNCTION OF UNANESTHETIZED GUINEA PIGS

Biological Activity Detected: Toxicity.

Principle: The method allows for measurement of respiratory and cardiovascular response of an unanesthetized guinea pig to a controlled test atmosphere.

Endpoints: Qualitative: ECG. Quantitative: Airway resistance, lung compliance, tidal volume, minute volume, breathing rate, heart rate.

Strengths: This system is most suitable for 1 to 3 hour exposures to gases, aerosols, drug response, complex pollutants, etc.; Each animal serves as its own control during testing.

Weaknesses: The system can be used for measuring response to a long term exposure where chronic breathing damage is expected. However, there is wide variation between animals. So for reliable results either a dramatic response is needed or a very large number of animals.

Status of Development: Being implemented.

Describe: System has been used for catalytically altered auto exhaust study, sulfur dioxide exposure study, and diesel engine auto exhaust study. Results are presently being evaluated.

Applications: Air; Multimedia.

Samples: Pure Chemicals: Sulfur dioxide. Complex Mixtures: Transportation Related - catalytically altered car exhaust, diesel auto emissions.

Duration: 5 hours/animal.

Cost/sample or chemical: $500/sample. This refers only to the test animals needed.

Interpretation: Reliable results require dramatic response differences.

Level of Complexity: 3.

OHEE Laboratory Involved: HERL-CIN.

Persons to Contact: M.J. Wiester, U.S. EPA, HERL-CIN, 26 W. St. Clair St., Cincinnati, OH 45268, (FTS 684-7424).

Grant/Contract Laboratory Involved and Principal Investigators: In-house.

Program Office Support: OHEE.

References: 1) Amdur, M.O., and J. Mead. Mechanics of Respiration in Unanesthetized Guinea Pigs. Am. J. Physiol., 192:364, 1958.

1148 ARTERIAL BLOOD GAS MEASUREMENT IN CONSCIOUS RATS

Biological Activity Detected: Toxicity.
Principle: Pollutant inhalation resulting in lung damage can decrease the ability of the animal to oxygenate the blood and remove CO_2.
Endpoints: Qualitative: N/A. Quantitative: Arterial blood P_{O_2}, P_{CO_2}, pH, bicarbonate.
Strengths: A sensitive indicator of lung damage; An important parameter as adequate P_{O_2} levels are necessary to support life; A sample can be collected quickly and fairly easily.
Weaknesses: Rats are the only small laboratory animal to which this method can be adapted; Extrapolation of results from rats to humans may be subject to criticism; Each animal can be used only once.
Status of Development: Validated.
Describe: Arterial blood gas measurements were carried out after 1, 3, 7, and 14 days exposure to either 0.75 or 1.0 ppm ozone. Arterial blood P_{O_2} declined in proportion to lung damage. A manuscript is in preparation.
Applications: Air; Water; Food.
Samples: Pure Chemicals: Sulfates, Oxidants, Trace metals.
Complex Mixtures: Ambient - air; Industrial - coal and rock dust; Energy Related - stack gas emission; Transportation Related - auto and diesel emission; Other - food and water born pollutants such as Paraquat.
Duration: 24 hours to 30 days.
Cost/sample or chemical: $200/animal.
Interpretation: A decrease in arterial P_{O_2} or an increase in P_{CO_2} indicates lung damage. The type of damage is not specified
Level of Complexity: 3.
OHEE Laboratory Involved: HERL-CIN, Laboratory Studies Division, Functional Pathology Branch.
Persons to Contact: W.E. Pepelko, U.S. EPA, HERL-CIN, 26 W. St. Clair St., Cincinnati, OH 45268, (FTS 684-7437).
Grant/Contract Laboratory Involved and Principal Investigators: N/A.
Program Office Support: OHEE.
References: 1) J. Appl. Physiol., 38:581-587, 1975. 2) Ann. Rev. Pharmacol. and Toxicol., 16:465-486, 1976. 3) Am. Rev. Resp. Dis., 113:531-559, 1976.

1149 INFECTIVITY MODEL

Biological Activity Detected: Toxicity.

Principle: Inhalation of a variety of gases and particulates has been shown to increase susceptibility to infectious pulmonary disease. Evaluation of the infectivity model used for these tests indicates that the model reflects the effects of a pollutant on a number of host defense systems, thereby increasing its sensitivity for detecting effects.

Endpoints: Qualitative: N/A. Quantitative: Mortality from laboratory-induced bacterial pneumonia is measured.

Strengths: The model is established for inhalation toxicology; Prior work has shown its exquisite sensitivity; The test is rapid; A battery of related follow-up tests are available; Whole animal inhalation exposures and dose-response studies are more directly applicable to standard setting and regulatory action.

Weaknesses: Relatively large amounts of pollutant sample are required for inhalation studies.

Status of Development: Validated.

Describe: The model has been successfully used for O_3, NO_2, Cd, Ni, sulfates and pollutant combinations. Even though a great potential exists it has not been used for screening purposes.

Applications: Air.

Samples: Pure Chemicals: Any chemical likely to reach gaseous exchange areas of the lung. Complex Mixtures: Ambient; Industrial; Energy Related; Transportation Related; Other - any other particulate, gas or combination of same.

Duration: 8 weeks/dose-response of 1 chemical.

Cost: $30,000 to $35,000/dose-response of 1 chemical.

Interpretation: Estimation of enhanced susceptibility to infectious diseases due to pollutant.

Level of Complexity: 3.

OHEE Laboratory Involved: HERL-RTP, Clinical Studies Division, Biomedical Research Branch.

Persons to Contact: D.E. Gardner, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2531).

Grant/Contract Laboratory Involved and Principal Investigators: IIT Research Institute, 10 West 35th St., Chicago, IL 60616, R. Ehrlich; Northrop Services, Inc., P.O. Box 12313, Research Triangle Park, NC 27709, B. Adkins.

Program Office Support: OHEE; OPP; OEMI; OTS.

References: 1) Coffin, D.L., and D.E. Gardner. Ann. Occup. Hyg., 15:219-234, 1972. 2) Coffin, D.L., et al. Envir. Hlth. Perspect., 13:11-15, 1976. 3) Ehrlich, R. Bacteriol. Rev., 30:604-614, 1966. 4) Ehrlich, R., et al. Internat. Conf. Photo. Oxid. Pollut. and Its Control, Proc. Vol 1, EPA-600/3-77-001a, 1977. pp. 565-574.

11410 IN-VIVO ALVEOLAR MACROPHAGE CYTOTOXICITY

Biological Activity Detected: Toxicity.

Principle: Cytotoxic effects of inhalation of environmental chemicals will be measured using isolated alveolar macrophages. Any alteration in these cells could increase the potential risk of respiratory infections.

Endpoints: Qualitative: N/A. Quantitative: The following measurements can be made: viability, phagocytic functioning, bacteriocidal activity, enzymatic profile, morphology and other biochemical parameters.

Strengths: A sensitive indicator of cytotoxicity using an in-vivo model system; Data generated quickly, which can be used to validate in-vitro cytotoxicity testing; Can serve as criteria for standard setting and regulatory purposes.

Weaknesses: Requires a substantial quantity of the test chemicals for generation of aerosols.

Status of Development: Validated.

Describe: The model has been successfully used for O_3, NO_2, Cd, Ni, Mn, cigarette smoke, and other metals.

Applications: Air.

Samples: Pure Chemicals: Particulates, Gases, Mists, Any chemical likely to reach gaseous exchange areas of the lung. Complex Mixtures: Ambient; Industrial; Energy Related; Transportation Related.

Duration: 8 weeks/dose-response of 1 chemical.

Cost: $30,000/dose-response of 1 chemical.

Interpretation: Depending upon the particular endpoint, alteration would indicate enhanced susceptibility to infectious disease or potential for alteration of lung tissue.

Level of Complexity: 2.

OHEE Laboratory Involved: HERL-RTP, Clinical Studies Division, Biomedical Research Branch.

Persons to Contact: D.E. Gardner, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2531).

Grant/Contract Laboratory Involved and Principal Investigators: IIT Research Institute, 10 West 35th St., Chicago, IL 60616, C. Aranyi; U. of California, Davis, CA 95616, E. Goldstein; Southwest Research Institute, San Antonio, Texas 78284, E. Gause.

Program Office Support: OHEE; OPP; OEMI; OTS.

References: 1) Gardner, D.E., et al. J. Bacteriol., 98:1041-1043, 1969. 2) Hurst, D.J., et al. J. Reticuloendothel. Soc., 8:288-300, 1970. 3) Bingham, E. Arch. Envir. Hlth., 25:406-414, 1972. 4) Gardner, D.E. Thesis, U. of Cincinnati, 1971. 5) Warshauer, D., et al. J. Lab. Clin. Med., 83:228-240, 1974. 6) Kass, E.H., et al. Bacteriol. Rev., 30:488-497, 1966. 7) Kim, M., et al. J. Infect. Dis., 133:310-320, 1976.

CONTENTS: TEST SYSTEMS

1150 In-Vivo Systems/Neurobehavioral. 47

  1151 Integrated System: Neurobehavioral Toxicological Assessment 48

  1152 Integrated System: The Effects of Selected Organic Contaminants in Drinking Water on the Functions of the Reproductive, Nervous, and Immune Systems. 49

  1153 Computer Automated Analysis of Patterned Behavior in the Primate. 51

  1154 Computer Automated Analysis of Learning and Memory in the Primate. 52

  1155 Integrated System: Behavioral Analysis of Rats — Developmental, Locomotor, Exploratory, and Learned Behavior. 53

  1156 Integrated System: Instrumental Methods of Detecting Functional and Metabolic Damage to the Nervous System. 54

1200 Genotoxicity. 57

  1210 Mutagenesis. 57

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1151 INTEGRATED SYSTEM: NEUROBEHAVIORAL TOXICOLOGICAL ASSESSMENT

Biological Activity Detected: Toxicity.

Principle: A battery of tests are being implemented which evaluate various aspects of CNS functions.

Endpoints: Qualitative: Evaluate CNS Function. Qualitative: These include: locomotor activity, neuromotor function, CNS excitability, learning and memory.

Strengths: Provides rapid and sensitive broad spectrum evaluation of CNS function.

Weaknesses: Requires skilled personnel; Real possibility of false negatives.

Status of Development: Validated.

Describe: This battery has been implemented and is currently being validated using known neurotoxins.

Applications: Multimedia.

Samples: Pure Chemicals: Heavy metal, Pesticides, Nonionizing radifilm. Complex Mixtures: N/A.

Duration: Test: 2 weeks, assuming acute exposure; Analysis: 3 weeks.

Cost: $2,000/sample, assuming acute exposure.

Interpretation: The profile of change in the various functional tests provide information on neurotoxicity of test compounds.

Level of Complexity: 3.

OHEE Laboratory Involved: HERL-RTP, Environmental Biology Division, Neurobiology Branch.

Persons to Contact: L. Reiter, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2671).

Grant/Contract Laboratory Involved and Principal Investigators: N/A.

Program Office Support: OHEE.

References: 1) Reiter, L., et al. Residential Maze. Envir. Hlth. Perspect., 12:119-123, 1975. 2) Archer, J. Anim. Behav., 21:205-235, 1973. 3) Dunham, N.W., and T.S. Miya. Roto Rod. J. of Amer. Pharmaceu. Asso., 46(3):208-209, 1957. 4) Gait, S., R. Rushton, and H. Stellberg. Anim. Behav. and Drug Action, 207-223, 1964. 5) Tremors, R.R., G.K. Chalmers, and W. Yim. Proc. Soc. Exp. Biol. Med., 109:202-205, 1962. 6) Hornston, M. Startle Reflex. Physio. and Rev., 3:839-844, 1968. 7) Reiter, L., et al. Passive Avoidance Test. Toxicol. and Appl. Pharm., 25:582-588, 1973. 8) Miczek, K., and H. Barr, II. Social Behavior. Behav. Pharmacol., 176-257, 1976.

1152 INTEGRATED SYSTEM: THE EFFECTS OF SELECTED ORGANIC CONTAMINANTS IN DRINKING WATER ON THE FUNCTIONS OF THE REPRODUCTIVE, NERVOUS, AND IMMUNE SYSTEMS

Biological Activity Detected: Reproductive, immuno-, neuro-, and behavioral toxicity; Mutagenicity.

Principle: Determine effects of experimental exposure to certain selected organic contaminants on: 1) Immune system and host resistance capabilities; 2) Neurochemical processes and dynamics in the brain; 3) Behavior; 4) Male reproductive function (including dominant lethal mutagenicity assay); 5) General toxicity endpoints (lethality, body-organ weights, hematology, etc.).

Endpoints: Qualitative: N/A. Quantitative: 1) Immune response and host resistance: Humoral - serum antibody production to S. aureus; Cell mediated - response to C. parvum; RES activity - global phagocyt. index (vasc. clearance) and tissue distribution of ^{14}C S. aureus; Susceptibility to pathogens (bact., virus, fungus) and transplant tumor; 2) Brain neurochemistry and dynamics: In-vivo and in-vitro (synaptosome) systems; Endogenous levels, uptake, release, metabolism...in Norepinephrine, dopamine, serotonin and AcCh systems; 3) Behavioral toxicology: Operant behavior - scheduled and learned performance; Learning ability; Behavior development; Other - elem. screen, spont. mot. activ., visual, swim, maze, etc; 4) Reproductive performance and dom. lethal mutagen. asso.: Antifertility, reversibility, mutagenic potential, mode of action, penetration of BT barrier (in-vivo); Penetration mechanism, spermatic nucleo/protein synth. spermatic uptake (in-vitro); 5) Preliminary and range finding shorter term toxicology: Lethality, body/organ weights, hematology, etc.

Strengths: Provides much information on several toxicologic aspect areas in coordinated manner; Readiness reduces time turnaround.

Weaknesses: Expensive.

Status of Development: Being implemented.

Describe: The tests are being implemented. Some are still developmental, but most are validated. Current work applicable to and aimed at drinking water contaminants, but techniques and inferences applicable to other media.

Applications: Water.

Samples: Pure Chemicals: Trihalomethanes; Benzenes; Ethers; Phenyls; Pesticides; PCB; Dioxin; 2, 4-DNT; (Many chemicals, but not all are under test by all the test systems under present project). Complex Mixtures: Not now under test but could be implemented.

Duration: Existing grants, about 2 years, covering multiple chemical; Individual tests involve short (1 to several days) to long-term (3 months) exposures.

Cost/sample or chemical: Approx. $120,000 for the entire integrated multidisciplinary workup involving multiple dose levels and exposure periods lasting up to a year for completion of all phases. However, application of selected portions, reduced exposures or fewer dose levels, etc., would be less expensive.

1152 INTEGRATED SYSTEM: THE EFFECTS OF SELECTED ORGANIC CONTAMINANTS IN DRINKING WATER ON THE FUNCTIONS OF THE REPRODUCTIVE, NERVOUS, AND IMMUNE SYSTEMS (continued)

Interpretation: Toxicity interpreted in terms of potential human health hazards.

Level of Complexity: Complexity levels range from 3 to 5.

OHEE Laboratory Involved: HERL-CIN, Laboratory Studies Division, Toxicologic Assessment Branch.

Persons to Contact: K.I. Campbell, U.S. EPA, HERL-CIN, 26 W. St. Clair St., Cincinnati, OH 45268, (FTS 684-7481).

Grant/Contract Laboratory Involved and Principal Investigators: Medical College of Virginia, Richmond, VA 23298: J.F. Borzelleca, Project Manager (Coordinator) and Reproduction Studies; A.E. Munson, Immune Systems, etc.; R.L. Balster, Behavioral Toxicology; W.L. Dewey, Neurochemistry.

Program Office Support: ORD.

References: 1) Grant document and report: R804701. 2) Grant document and report: R804290. 3) Deichmann, W., et al. Toxicol. and Appl. Pharmacol., 5:201, 1963. 4) Ball, H. J. Nat. Can. Inst., 44:1070, 1966. 5) Szakol, L., and H. Hanna. Nat. Can. Inst. Monographo., 35:173, 1972 6) For additional related references, contact investigator.

1153 COMPUTER AUTOMATED ANALYSIS OF PATTERNED BEHAVIOR IN THE PRIMATE

Biological Activity Detected: Toxicity.

Principle: Animal behavior is structured (patterned) and chemicals which affect CNS function will disrupt this patterned behavior.

Endpoints: Using closed-circuit TV and on-line computing, various components of primate behavior are defined and measured for frequency, duration, and sequences. Qualitative: N/A. Quantitative: Frequency of various motor items in experimental period; Duration of various motor items per occurrence; Sequencing of behavior.

Strengths: Should provide sensitive index of neurotoxicity which is fully automated and applicable to a wide range of pollutant testing; Also no pre-training is required.

Weaknesses: Expensive; Specialized equipment is required.

Status of Development: Developmental.

Describe: System has been developed and is being tested with known psychoactive drugs.

Applications: Multimedia.

Samples: Pure Chemicals: Heavy metals. Complex Mixtures: N/A.

Duration: Test: 1 week, assuming acute exposure; Analysis: 2 weeks.

Cost: $200/subject.

Interpretation: Changes in frequency, duration, or patterning of behavior are indicative of neurotoxicity.

Level of Complexity: 3.

OHEE Laboratory Involved: HERL-RTP, Environmental Biology Division, Neurobiology Branch.

Persons to Contact: L. Reiter, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2671).

Grant/Contract Laboratory Involved and Principal Investigators: Iowa State U., Ames, IA 50010, W.E. Lloyde.

Program Office Support: OEMI.

References: 1) Norton, S. Physiol. Behav., 11:181-186, 1973. 2) Norton, S. J. Theoret. Biol., 21:229-243, 1962.

1154 COMPUTER AUTOMATED ANALYSIS OF LEARNING AND MEMORY IN THE PRIMATE

Biological Activity Detected: Toxicity.

Principle: Chemicals affecting CNS function may interfere with the learning process. The fact that primates rely heavily on the visual sensory modality is utilized to determine pollutant effects on visual discrimination learning.

Endpoints: Qualitative: N/A. Quantitative: Two choice non-spatial visual discrimination tasks examine animals' ability to distinguish between different visual patterns; Delayed response task: evaluates memory coordination utilizing visual stimuli.

Strengths: Provides system index of chemical effects on learning and memory; Provides information on behavioral effects in primates.

Weaknesses: Expensive; Requires skilled personnel to perform test; Used only for toxicity testing of pure compounds when specific information on primates is required.

Status of Development: Developmental.

Describe: System has been developed and is being tested with psycho-active drugs.

Applications: Multimedia.

Samples: Pure Chemicals: Heavy metals. Complex Mixtures: N/A.

Duration: Test: 6 weeks, assuming acute exposure; Analysis: 2 weeks.

Cost: $200/subject.

Interpretation: Alterations in performace are indicative of neurotoxicity.

Level of Complexity: 3.

OHEE Laboratory Involved: HERL-RTP, Environmental Biology Division, Neurobiology Branch.

Persons to Contact: L. Reiter, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2671).

Grant/Contract Laboratory Involved and Principal Investigators: Iowa State U., Ames, IA 50010, W.E. Lloyde.

Program Office Support: OEMI.

References: 1) Fletcher, H.J. The Delayed-Response Problem. In: Behavior of Nonhuman Primates, A.M. Schrier, H.F. Harlow, and F. Stollnitz, eds. Academic Press, New York, 1965. pp. 129-165. 2) Miles, R.C. Discrimination-Learning Sets. In: Behavior of Nonhuman Primates, A.M. Schrier, H.F. Harlow, and F. Stollnitz, eds. Academic Press, New York, 1965. pp. 53-54.

1155 INTEGRATED SYSTEM: BEHAVIORAL ANALYSIS OF RATS — DEVELOPMENTAL, LOCOMOTOR, EXPLORATORY, AND LEARNING BEHAVIOR

Biological Activity Detected: Toxicity; Behavioral.

Principle: Various levels of lead are known to affect the maturation of energy metabolism in the cerebral cortex of rats. Studies have shown that delays in the development of the nervous system may affect behavioral responses observable in both the young and adult animal. Specific affects need to be tested on the above listed indices.

Endpoints: Qualitative: Expression of learned behavior through use of a water T-Maze - percentage of correct trials and amount of time to reach criteria; Expression of locomotor activity and exploratory behavior through the use of a Berylene Box - primarily a measurement of frequency and duration spent on each parameter; Expression of developmental behavior through locomotor activity and use of ultrasonics - the measurement of general activity patterns and monitoring of communication abilities dependent upon development. Quantitative: Maze activity; Benylene box; Generalized and specific locomotor activity; Frequency and duration of ultrasonic vocalizations.

Strengths: Should provide sensitive behavioral tests which can then be correlated with physiological data collected in previous studies.

Weaknesses: Requires specialized instrumentation for testing; Time-consuming to run the battery of tests for each designated dosage level.

Status of Development: Being implemented.

Describe: Most of the instrumentation has been constructed and subjects are being treated with designated lead doses.

Applications: Water.

Samples: Pure Chemicals: Trace metals, Organic chemicals.

Complex Mixtures: Industrial; Energy Related.

Duration: 1 to 3 months.

Cost: Not yet established.

Interpretation: Altered physiological or neurological function as a result of treatment chemical would indicate toxicity.

Level of Complexity: 3.

OHEE Laboratory Involved: HERL-CIN, Laboratory Studies Division, Toxicological Assessment Branch.

Persons to Contact: R.J. Bull, U.S. EPA, HERL-CIN, 26 W. St. Clair St., Cincinnati, OH 45268, (FTS 684-7213).

Grant/Contract Laboratory Involved and Principal Investigators: N/A.

Program Office Support: OHEE; OWHM.

References: 1) Reiter, L.W., et al. Envir. Hlth. Perspect., 12:119-123, 1975. 2) Sobotka, T.J., et al. Toxicol., 5:175-191, 1975. 3) Brown, D.R. Toxicol. Appl. Pharmacol., 32:628-637, 1975. 4) Padich, R., et al. Pharmacol. Biochem. Behav., 6:371-375, 1977. 5) Fox, D.A., et al. Toxicol. Appl. Pharmacol., 40:449-461, 1977. 6) Robbins, T., et al. Psychopharmacologia, 28:155-164, 1972. 7) Amsel, A., et al. Science, 197:786-788, 1977.

1156 INTEGRATED SYSTEM: INSTRUMENTAL METHODS OF DETECTING FUNCTIONAL AND METABOLIC DAMAGE TO THE NERVOUS SYSTEM

Biological Activity Detected: Toxicity.

Principle: Increased functional activity of a tissue requires energy. Consequently, if a tissue's functional activity is stimulated, ATP is hydrolyzed to ADP and P_i, which in turn stimulates oxidation of substrate and resynthesis of ATP. These metabolic changes may be observed as increases in oxygen consumption, substrate utilization, or as metabolic transients induced in the electron carriers directly in tissues, in-vitro. The kinetics of these metabolic responses to stimulation have been shown sensitive to a wide variety of chemical agents with varying mechanisms of action with both in-vitro and in-vivo treatments.

Endpoints: To this point in time, an endpoint has only been developed for brain tissue. Responses are measured in response to electrical pulses (10 s) or elevation in K concentrations (3 to 30 mM). Qualitative: N/A. Quantitative: Transient redox changes in NAD(P)H, fp, cyt a, b, c.; Substrate utilization; Oxygen consumption; Lactic acid output; Neurotransmitter release; Amino acid metabolism.

Strengths: In-vitro results may be directly confirmed in-vivo with the same parameters; Applicable to a wide variety of mechanisms; Applicable to all aerobic tissues; Involves measurement of the kinetics of going from a resting to an excited state rather than the steady state greatly increasing sensitivity; Applicable to very small tissue samplers (2 to 3 mg).

Weaknesses: Does not lend itself to immediate identification of mechanisms unless there is a direct effect on energy metabolism proper.

Status of Development: Validated.

Describe: The test system has been validated with a wide variety of inhibitors of energy metabolism and membrane active compounds. In-vitro and in-vivo treatments with lead, methyl mercury, and alkylation compounds indicate equivalent or more sensitive measures of effect than other parameters which have been applied to these problems.

Applications: Multimedia.

Samples: Pure Chemicals: All classes. Complex Mixtures: Industrial; Energy Related; Transportation Related.

Duration: 2 weeks to 3 months.

Cost: Not yet established.

Interpretation: This is a general system for determining neurotoxicity. It is capable of detecting non-specific damage to a variety of systems (e.g., decreased membrane excitability, altered responses to neurotransmitters, direct effects on energy metabolism). It has also been used to detect delays in brain development, which were subsequently confirmed by morphologic and behavioral methods.

Level of Complexity: 3.

OHEE Laboratory Involved: HERL-CIN, Laboratory Studies Division, Toxicological Assessment Branch.

1156 INTEGRATED SYSTEM: INSTRUMENTAL METHODS OF DETECTING FUNCTIONAL AND METABOLIC DAMAGE TO THE NERVOUS SYSTEM (continued)

Persons to Contact: R.J. Bull, U.S. EPA, HERL-CIN, 26 W. St. Clair St., Cincinnati, OH 45268, (FTS 684-7213).

Grant/Contract Laboratory Involved and Principal Investigators: In-house.

Program Office Support: OHEE.

References: 1) Bull, R.J., and A.J. Trevor. J. Neurochem., 19:999-1009, 1972. 2) Bull, R.J., and A.J. Trevor. J. Neurochem., 19: 1011-1022, 1972. 3) Cummins, J.T., and R. Bull. Biochem. Biophys. Acta, 253:29-38, 1971. 4) Bull, R.J., and J.T. Cummins. J. Neurochem., 21:923-937, 1973. 5) Bull, R.J., and S.D. Lutkenhoff. Neuropharmacol., 14:351-359, 1975. 6) Bull, R.J. J. Neurochem., 26:149-156, 1976. 7) Bull, R.J., P.M. Stanaszek, J.J. O'Neill, and S.D. Lutkenhoff. Envir. Hlth. Perspect., 12: 89-95, 1975.

CONTENTS: TEST SYSTEMS

1200 Genotoxicity. 57

1210 Mutagenesis 57

1211 _Salmonella typhimurium_ (Ames) 58

1212 _Escherichia coli_ (WP2). 60

1213 _Saccharomyces cerevisiae_, _Schizosaccharomyces pombe_, Forward and Reverse Mutation. 61

1214 Body Fluid Analysis 62

1215 Bacterial Plasmids. 63

1216 Mouse Lymphoma (L5178Y) 64

1217 Chinese Hamster Ovary Cells (CHO) Drug Resistance. 65

1218 Chinese Hamster Cells (CHO) Nutritional Competency. 66

1219 Chinese Hamster Lung Cells (V79). 67

12110 _Drosophila melanogaster_, Sex Linked Recessive Lethal. 68

12111 _Tradescantia_ Stamen Hair. 69

12112 Maize Waxy Locus Assay. 70

12113 In-Vivo Cytogenetics in Mice. 71

1220 DNA Damage. 73

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1211 SALMONELLA TYPHIMURIUM (AMES)

Biological Activity Detected: Mutagenicity; Presumptive oncogenicity.

Principle: Histidine dependent strains of Salmonella genetically engineered to increase their sensitivity and specificity, are exposed to a test substance in the presence of mammalian metabolic activating enzymes. The formation of bacterial colonies in a histidine-free medium after treatment is considered a criteria of the effectiveness of a test substance as a mutagen.

Endpoints: Qualitative: Spot test and plate incorporation test are considered to be qualitative in nature. In spot tests no attempt is made to count the number of colonies per plate. In plate incorporation the number of colonies are counted but not expressed as a fraction of survival. Quantitative: The induced mutational frequencies may be expressed on the basis of units of test material and survival in suspension.

Strengths: Genetically well-characterized system; Rapid; Inexpensive; Well validated as a test for gene mutation; Works well with in-vitro metabolizing microsome fractions; Can be used as indicator organism in host-mediated assays.

Weaknesses: Reverse mutation assay requiring several strains to permit detection of a broad spectrum of compounds; Requires metabolic activation; Lacks pharmacological relevance; Prokaryotic organization of genetic material.

Status of Development: Validated.

Describe: N/A.

Applications: Multimedia.

Samples: Pure Chemicals: All major classes of chemicals except metals and hormones. Complex Mixtures: Ambient - air particulates, drinking water, and water concentrates; Industrial - effluents; Energy Related - alternate effluents, shale; Transportation Related - auto/truck fuels; Other - human body fluids, extracts from crops treated with sludge.

Duration: 3 weeks/study.

Cost: $300 to $650/compound for plate test; $1,000 to $1,200/compound for suspension test.

Interpretation: The growth of colonies in a histidine-deficient medium indicates genetic alteration.

Level of Complexity: 2.

OHEE Laboratory Involved: HERL-RTP, Environmental Toxicology Division, Biochemistry Branch, Cellular Biology Section; HERL-CIN, Field Studies Division, Toxicological Assessment Branch; ERL-GB; National Center for Toxicological Research, Division of Mutagenesis, Somatic Cell Section.

Persons to Contact: J.L. Huisingh, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2948); L. Claxton, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2942); M.D. Waters, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2693); J.P. Bercz, U.S. EPA, HERL-CIN, 26 W. St. Clair St., Cincinnati, OH 45268, (FTS 684-7432); H.R. Pahren, U.S. EPA, HERL-CIN, 26 W. St. Clair St., Cincinnati, OH 45268, (FTS 684-7217); L.J. McCabe, U.S. EPA, HERL-CIN, 26 W. St. Clair St., Persons to Contact (continued): Cincinnati, OH 45268, (FTS 684-7211); J.F. Stara, U.S. EPA, HERL-CIN, 26 W. St. Clair St., Cincinnati, OH 45268, (FTS 684-7407); N. Richards, U.S. EPA, ERL-GB, Sabine Island, Gulf Breeze, FL 32561, (FTS 686-9011); E. Lazear, NCTR, Jefferson, AR 72079, (FTS 740-4573); D.A. Casciano, NCTR, Jefferson, AR 72079, (FTS 740-4495).

Grant/Contract Laboratory Involved and Principal Investigators: Stanford Research Institute, Menlo Park, CA, V.F. Simmons; Litton Biometics, Inc., Nicholson Lane, Kensington, MD, D.T. Brusick; U. of Cincinnati Medical Center, J. Loper; Louisiana State U. Medical School, W. Pelon; U. of Missouri, Columbia, MO, C. Marianseld; U. of West Florida, J. Bazlis; U. of Texas, Medical Branch, Galveston, TX 77550, M. Legator.

Program Office Support: OHEE; OPP; OEMI; OTS.

References: 1) Ames, B.N., et al. Mutation Res., 31:347-364, 1975. 2) McCann, et al. Proc. Natl. Acad. Sci., 70:782-786, 1975. 3) McCann, and B.N. Ames. Proc. Natl. Acad. Sci., 73:950-954, 1976.

1212 ESCHERICHIA COLI (WP2)

Biological Activity Detected: Mutagenicity.

Principle: Tryptophan dependent strains of Escherichia coli genetically engineered to increase their sensitivity and specificity, are exposed to a test substance in the presence of mammalian metabolic activating enzymes. The formation of bacterial colonies in a tryptophan-free medium after treatment indicates the effectiveness of a test substance as a mutagen.

Endpoints: Qualitative: Growth in a tryptophan-free medium. Quantitative: N/A.

Strengths: Rapid; Inexpensive; Well validated test for gene mutation; Works well with in-vitro metabolizing microsomal enzymes; Can be used as indicator organism in host-mediated assays.

Weaknesses: Reverse mutation assay requiring several strains to permit detection of a broad spectrum of compounds; Requires metabolic activation; Lacks pharmacological relevance; Prokaryotic organization of genetic material; Not as well characterized nor as sensitive as Salmonella/microsome assay (1211). It detects only base pair substitutions.

Status of Development: Validated.
Describe: N/A.

Applications: Multimedia.

Samples: Pure Chemicals: All major classes of chemicals except metals and hormones. Complex Mixtures: Ambient - air particulates, drinking water, and water concentrates; Industrial - effluents; Energy Related - alternate effluents, shale; Transportation Related - auto/truck fuels; Other - human body fluids, extracts from crops treated with sludge.

Duration: 3 weeks.

Cost: $450/chemical.

Interpretation: The growth of tester strains in a tryptophan free medium after treatment with a test substance indicates mutation.

Level of Complexity: I.

OHEE Laboratory Involved: HERL-RTP, Environmental Toxicology Division, Biochemistry Branch, Cellular Biology Section.

Persons to Contact: M.D. Waters, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2693); L. Claxton, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2942); S.S. Sandhu, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2693); J.L. Huisingh, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2948).

Grant/Contract Laboratory Involved and Principal Investigators: Stanford Research Institute, Menlo Park, CA 94205, V.F. Simmons.

Program Office Support: OHEE; OPP.

References: 1) Bridges, B.A. Lab Practice, 21:413-416, 1972.
2) Bridges, B.A., et al. Mutation Res., 19:295-303, 1973.
3) Bridges, B.A., et al. Chem/Biol. Interactions, 5:77-84, 1972.

1213 SACCHAROMYCES CEREVISIAE, SCHIZOSACCHAROMYCES POMBE, FORWARD AND REVERSE MUTATION

Biological Activity Detected: Mutagenicity.

Principle: Gene mutation is detected by loss of function resulting in nutritional requirements or resistance to toxic chemicals and shift in color of cell colonies.

Endpoints: Qualitative: Growth in a selective medium; Change in colony pigmentation. Quantitative: Mutation frequencies may be adjusted for cytotoxic effects.

Strengths: Both forward and reverse mutation can be studied; Eukaryotic organization of genetic material; Fast; Relatively inexpensive; Cells can be cultured as haploids; Fairly wide spectrum of genetic events can be scored; Can be used as indicator organism in host-mediated assays.

Weaknesses: Requires exogenous metabolic activation which has not worked well with yeast systems; Lacks pharmacological relevance; Chromosomes are too small to permit direct cytological observation; Relatively insensitive to some chemicals.

Status of Development: Validated.

Describe: N/A.

Applications: Multimedia.

Samples: Pure Chemicals: Alkylating agents, Halogenated hydrocarbons, Polycyclics, Carbamates. Complex Mixtures: Ambient - water concentrated; Other - extracts from crops treated with sludge, human body fluids.

Duration: 3 weeks.

Cost: $400 to $700/compound, depending on the test.

Interpretation: The appearance of pigmented colonies and growth in selective mutation after treatment with test compound indicates mutation.

Level of Complexity: I.

OHEE Laboratory Involved: HERL-RTP, Environmental Toxicology Division, Biochemistry Branch, HERL-CIN, Field Studies Division, Toxicological Assessment Branch.

Persons to Contact: M.D. Waters, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2693); S.S. Sandhu, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2693); J.P. Bercz, U.S. EPA, HERL-CIN, 26 W. St. Clair St., Cincinnati, OH 45268, (FTS 684-7432); H.R. Pahren, U.S. EPA, HERL-CIN, 26 W. St. Clair St., Cincinnati, OH 45268, (FTS 684-7217).

Grant/Contract Laboratory Involved and Principal Investigators: Stanford Research Institute, Menlo Park, CA 94205, V.F. Simmons.

Program Office Support: OHEE; OPP; OEMI.

References: 1) Zimmermann, F.K. In: Chemical Mutagens: Principles and Methods for Their Detection. Vol. 3. A. Hollaender, ed. Plenum Press., NY, 1973. pp. 209-239. 2) Parry, J.M. Mutation Res., 46(3):165-176, 1977. 3) Brusick, D.J., and V.W. Mayer. Envir. Hlth. Perspect., 6:83-96.

Biological Activity Detected: Mutagenicity.

Principle: Promutagens which need mammalian metabolic activation are biotransformed in the intact animal and are tested for mutagenic activity in Salmonella, yeast, and Chinese hamster ovary cell test systems.

Endpoints: Qualitative: Appearance of revertant colonies in a selective medium. Quantitative: The number of prototrophic colonies in a histidine deficient medium, adjusted for cytotoxic effects of the test chemical.

Strengths: Combines in-vivo metabolic activation with the in-vitro microbial test system.

Weaknesses: Limited number of bacteria exposed; Recovery of bacteria is problematic; Exposure time has not been standardized; Difficult to quantitate the response.

Status of Development: Being implemented.

Describe: The basic experimental procedure for pure chemicals has been established. But protocol for testing for mixtures has not been developed. Furthermore, the test system needs validation by testing a wide variety of chemicals.

Applications: Multimedia.

Samples: Pure Chemicals: Mycotoxins, Nitrosamines, Aromatic amines, Aromatic hydrocarbons. Complex Mixtures: Not yet tested.

Duration: 4 weeks.

Cost: $1,000 to $1,200/chemical.

Interpretation: The positive response indicates the ability of the test chemical to be transformed into reactive electrophile by the intact mammalian metabolic activation system.

Level of Complexity: 3.

OHEE Laboratory Involved: HERL-RTP, Environmental Toxicology Division, Biochemistry Branch; HERL-CIN, Field Studies Division, Toxicological Assessment Branch.

Persons to Contact: M.D. Waters, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2693); S.S. Sandhu, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2693); L. Claxton, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2942); J.F. Stara; U.S. EPA, HERL-CIN, 26 W. St. Clair St., Cincinnati, OH 45268, (FTS 684-7407).

Grant/Contract Laboratory Involved and Principal Investigators: U. of Texas, Medical Branch, Galveston, TX 77550, M. Legator. Stanford Research Institute, Menlo Park, CA 94205, V.F. Simmons.

Program Office Support: OHEE; OPP; OEMI.

References: 1) Legator, M., et al. Mutation Res. 26:456, 1974.
2) Legator, M., et al. In: Chemical Mutagens: Principles and Methods for Their Detection. Vol. 4. A. Hollander, ed. Plenum Press, NY, 1976. pp. 171-190.

1215 BACTERIAL PLASMIDS

Biological Activity Detected: Mutagenicity.

Principle: Purified plasmid DNA is exposed to potential mutagen in a cell-free system and is then analyzed for ability to infect a host bacterium.

Endpoints: Qualitative: Ability of treated plasmid DNA to infect bacterial cells. Quantitative: The number of colonies produced per unit of test materials.

Strengths: May avoid problems of extreme cytotoxicity of many chemicals since the exposure is in a cell-free system; Rapid; Economical.

Weaknesses: Problems with exposing purified DNA; Still early in developmental stage; Requires exogenous metabolic activation systems.

Status of Development: Developmental.

Describe: Initial pilot work with MNNG is encouraging.

Applications: Multimedia.

Samples: Pure Chemicals: Organics. Complex Mixtures: Ambient; Industrial; Energy Related; Transportation Related; Other.

Duration: 1 to 2 days.

Cost: Undetermined.

Interpretation: Loss of infectivity suggests damage to DNA.

Level of Complexity: 2.

OHEE Laboratory Involved: HERL-CIN, Laboratory Studies Division, Toxicological Assessment Branch.

Persons to Contact: N. Clarke, U.S. EPA, HERL-CIN, 26 W. St. Clair St., Cincinnati, OH 45268, (FTS 684-7411); B. Daniel, U.S. EPA, HERL-CIN, 26 W. St. Clair St., Cincinnati, OH 45268, (FTS 684-7482).

Grant/Contract Laboratory Involved and Principal Investigators: N/A.

Program Office Support: OHEE.

References: Not yet available.

1216 MOUSE LYMPHOMA (L5178Y)

Biological Activity Detected: Mutagenicity.

Principle: Forward mutation from thymidine kinase competency ($TK^{+/-}$) to thymidine kinase incompetency ($TK^{-/-}$), resulting in resistance to the base analogs BUdR or TFT.

Endpoints: Gene mutation. Qualitative: Formation of cell colonies in a selective medium. Quantitative: Induced mutation frequencies are based on cell survival and cloning efficiency.

Strengths: Both forward and reverse mutation can be measured; Cell will grow in suspension culture; Cells have short generation time; Cells have stable, near-diploid chromosome number; High plating efficiency; High recovery of mutant cells.

Weaknesses: Mutation measured at a single locus; Requires metabolic activation; Requires additional validation; Lacks pharmacological relevance; PPLO contamination is a serious problem and the cells must be continuously monitored to ensure that they are PPLO-free.

Status of Development: Being implemented.

Describe: Problems of expression time and metabolic activation have yet to be resolved. This bioassay has been contracted out for validation by the National Cancer Institute.

Applications: Multimedia.

Samples: Pure Chemicals: Alkylating agents, Halogenated hydrocarbons, Inorganic derivatives, N-Nitroso compounds, Metals, Mycotoxins. Complex Mixtures: Ambient; Industrial.

Duration: 3 weeks.

Cost: $3,000/compound.

Interpretation: Growth of heterozygous thymidine competent cells in a medium containing TFT or BUdR suggests mutation.

Level of Complexity: 4.

OHEE Laboratory Involved: HERL-RTP, Environmental Toxicology Division, Biochemistry Branch, Cellular Biology Section.

Persons to Contact: M.D. Waters, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2693); S.S. Sandhu, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2693); M.M. Brown, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2693).

Grant/Contract Laboratory Involved and Principal Investigators: Stanford Research Institute, Menlo Park, CA 94205, A. Mitchell.

Program Office Support: OHEE; OPP; OTS.

References: 1) Clive, D., and J.F.S. Spector. Laboratory Procedure for Assessing Specific Locus Mutations at the TK Locus in Cultured L5178Y Mouse Lymphoma Cells. Mutation Res., 31:17-29, 1975.

1217 CHINESE HAMSTER OVARY CELLS (CHO) DRUG RESISTANCE

Biological Activity Detected: Mutagenicity; Presumptive oncogenicity.

Principle: Forward mutation assay measuring drug resistance at hypoxanthine-guanine-phosphoribolyltransferase (HGPRT) locus. The HGPRT competent cells in presence of mammalian metabolic activation enzymes are exposed to a test substance. Induced frequency of HGPRT deficiency is determined using a selective medium containing base analogue 8-azaguanine (8 AZ) or 6-thioguanine (6 TH).

Endpoints: Gene mutation. Qualitative: Formation of cell colonies in a selective medium. Quantitative: Induced mutation frequencies are based on cell survival and cloning efficiency.

Strengths: Mammalian organization of genetic material; Forward mutation assay; Fast generation time; Stable karyotype; Easy to culture; May be particularly well-suited to quantitation as HGPRT locus mutants may not show replicative advantages/disadvantages over wild-type cells under nonselective conditions.

Weaknesses: Mutation measured at a single locus; Requires metabolic activation; Needs additional validation; Lacks pharmacological relevance; Fairly high spontaneous mutation rate; Long optimal expression period (7 days).

Status of Development: Being implemented.

Describe: Basic system with metabolic activation has been described. Several compounds representing diverse classes of chemicals have been tested. However, additional chemicals by at least two laboratories have to be tested before it could be considered as validated.

Applications: Multimedia.

Samples: Pure Chemicals: Alkylating agents, Nitrosamines, Organics, PNA's, Metallic compounds. Complex Mixtures: Not yet determined.

Duration: 3 weeks/compound.

Cost: $2,000 to $3,000/compound, including dose-response.

Interpretation: Growth of HGPRT competent cells in a medium containing 8 AZ suggests mutation.

Level of Complexity: 4.

OHEE Laboratory Involved: HERL-RTP, Environmental Toxicology Division, Biochemistry Branch; National Center for Toxicological Research, Division of Mutagenesis, Somatic Cell Section.

Persons to Contact: M.D. Waters, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2693); S.S. Sandhu, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2693); D.A. Casciano, NCTR, Jefferson, AR 27079, (FTS 740-4495).

Grant/Contract Laboratory Involved and Principal Investigators: Oak Ridge National Laboratory, Biology Division, P.O. Box Y, Oak Ridge, TN 37830, A. Hsie.

Program Office Support: OHEE.

References: 1) O'Neill, P.J., et al. Mutation Res., 45:91-101, 1977.
2) O'Neill, P.J., et al. Mutation Res., 45:103-109, 1977.

1218 CHINESE HAMSTER CELLS (CHO) NUTRITIONAL COMPETENCY

Biological Activity Detected: Mutagenicity.

Principle: Isolation of auxotrophic mutants using 5-bromodeoxyuridine (BUdR) and visible light as selective agents.

Endpoints: Gene and chromosomal mutation. Qualitative: Formation of auxotrophic cell colonies. Quantitative: Can be used to determine induced mutation frequencies based on cell survival and cloning efficiency.

Strengths: System is clean (survivors are either auxotrophs or they are not); Several loci are available for detection of genetic changes; Forward mutation assay with very low spontaneous mutation frequencies at available loci; Test populations can be easily freed of spontaneous auxotrophs by growing cells three days in minimal medium; Mammalian system in terms of organization of genetic materials.

Weaknesses: Assay very insensitive (95% of auxotrophs lost during selection to effects of starvation); Mutant identification is tedious and not amenable to screening; Experiments require five weeks to complete; Requires metabolic activation; Requires validation; Lacks pharmacological relevance; Nutritional mutants appear to be at a replicative disadvantage in mixed populations under nonselective conditions.

Status of Development: Developmental.
Describe: Improvement of technique is necessary for increased sensitivity. Also necessary is the addition of an in-vitro metabolic activation.

Applications: Multimedia.

Samples: Pure Chemicals: Alkylating esters, Base analogs, Nitrosamines, Acridine, Mustards, Heavy metals, Hydroxylamine.
Complex Mixtures: Energy Related - petroleum oil extracts; Transportation Related - jet fuel extracts; Other - ultraviolet radiation, x-rays.

Duration: 5 weeks.

Cost: $2,000/assay.

Interpretation: Test agents inducing significant numbers of auxotrophs may be regarded as potential mutagens/carcinogens for animals and man.

Level of Complexity: 4 to 5.

OHEE Laboratory Involved: ERL-NAR, Toxicology Branch, Genetic Toxicology Team.

Persons to Contact: A.R. Malcolm, U.S. EPA, ERL-NAR, South Ferry Rd., Narrangansett, RI 02882, (FTS 838-4843 X247 or X238).

Grant/Contract Laboratory Involved and Principal Investigators: N/A.

Program Office Support: OHEE.

References: 1) Kao, F.T., and T.T. Puck. Induction and Isolation of Auxotrophic Mutants in Mammalian Cells. In: Methods in Cell Biology. Vol. 3. D. Prescott, ed. Academic Press, NY, 1974. pp. 23-39.

1219 CHINESE HAMSTER LUNG CELLS (V79)

Biological Activity Detected: Mutagenicity.

Principle: Forward mutation assay measuring drug resistance at hypoxanthine-guanine-phosphoribolyltransferase (HGPRT) locus. The HGPRT competent cells in presence of mammalian metabolic activation enzymes are exposed to a test substance. Induced frequency of HGPRT deficiency is determined by using a selective medium containing base analogues 8-azaguanine (8 AZ).

Endpoints: Gene mutation. Qualitative: Formation of cell colonies in a selective medium. Quantitative: Induced mutation frequencies are based on cell survival and cloning efficiency.

Strengths: Mammalian organization of genetic material; Forward mutation assay; Fast generation time; Stable karyotype; Easy to culture; May be particularly well-suited to quantitation as HGPRT locus mutants may not show replicative advantages/disadvantages over wild-type cells under nonselective conditions.

Weaknesses: Mutation measured at a single locus; Requires metabolic activation; Requires additional validation; Lacks pharmacological relevance; Fairly high spontaneous mutation rate ($1^{-5} \times 10^{-6}$); Long optimal expression period (7 days).

Status of Development: Being implemented.

Describe: Needs additional validation.

Applications: Multimedia.

Samples: Pure Chemicals: N-Nitroso compounds, Alkylating agents, Primarily organics mixtures. Complex Mixtures: Ambient; Industrial; Energy Related.

Duration: 4 weeks.

Cost: $3,000.

Interpretation: Growth of the treated HGPRT competent cells in a selective medium suggests genetic change.

Level of Complexity: 4.

OHEE Laboratory Involved: HERL-RTP, Environmental Toxicology Division, Biochemical Branch; HERL-CIN, Field Studies Division, Toxicological Assessment Branch.

Persons to Contact: M.D. Waters, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2693); S.S. Sandhu, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2693); N.E. Kowal, U.S. EPA, HERL-CIN, 26 W. St. Clair St., Cincinnati, OH 45268, (FTS 684-7477).

Grant/Contract Laboratory Involved and Principal Investigators: Stanford Research Institute, Menlo Park, CA 94205, A. Mitchell; Gulf South Research Institute, P.O. Box 26518, New Orleans, LA 70186, N. Gruener.

Program Office Support: OHEE.

References: 1) Krahn, D.F., and C. Heidelburger. Proc. Natl. Acad. Sci., 73:188-192, 1977. 2) Artlett, et al. Mutation Res., 33: 261-278, 1975.

12110 DROSOPHILA MELANOGASTER, SEX LINKED RECESSIVE LETHAL

Biological Activity Detected: Mutagenicity.

Principle: Wild type males are treated with the test chemical and mated with untreated females with a marked chromosome. F_1 females are sib-mated and the progeny are scored for the presence of x-linked recessive lethals.

Endpoints: Qualitative: Change in sex ratios in F_2 generation. Quantitative: Point mutations and small delections may be scored in germ cells.

Strengths: Higher organism, genetically well characterized; Multiple loci available for detection of genetic alterations; Some metabolic processes similar to that of mammals; Small number of large chromosomes; Broad spectrum of genetic events can be detected and scored; Wealth of mutant strains makes possible detailed analysis of induced genetic changes.

Weaknesses: Short life span makes organism unsuitable for chronic exposure studies; Limited use for testing pesticides due to extreme toxicity.

Status of Development: Validated.

Describe: N/A.

Applications: Multimedia.

Samples: Pure Chemicals: Alkylating agents, Nitrosamines, Halogenated ethers. Complex Mixtures: Protocols not yet developed.

Duration: 4 to 6 weeks.

Cost: $4,000 to $6,500.

Interpretation: Change in the sex ratio in the F_2 progeny of the test population indicates mutation.

Level of Complexity: 4.

OHEE Laboratory Involved: HERL-RTP, Environmental Toxicology Division, Biochemistry Branch, Cellular Biology Section.

Persons to Contact: M.D. Waters, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2693); S.S. Sandhu; U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2693).

Grant/Contract Laboratory Involved and Principal Investigators: Stanford Research Institute, Menlo Park, CA 94205, A. Mitchell.

Program Office Support: OHEE.

References: 1) Vogel, E., and F.H. Sobels. The Function of *Drosophila* in Genetic Toxicology Testing. In: Chemical Mutagens: Principles and Methods for Their Detection. Vol. 4. A. Hollaender, ed. Plenum Press, NY, 1976. pp. 93-132. 2) Sobels, F.H., and E. Vogel. The Capacity of *Drosophila* for Detecting Relevant Genetic Damage. Mutation Res., 41:95-106, 1976. 3) Legator, M.S., and S. Zimmering. Gen. Toxicol. Ann. Rev. Pharmacol., 387-408, 1975.

12111 *TRADESCANTIA* STAMEN HAIR

Biological Activity Detected: Mutagenicity.

Principle: Mutation in petals and stamen hair in clones heterozygous for flower color is detected as a change in pigmentation.

Endpoints: *Qualitative*: Change in stamen hair cells' color from blue to pink. *Quantitative*: Mutational events per stamen hair and dose-response relationship can be established.

Strengths: Can detect broad spectrum of genetic events; Can be used to monitor in situ environment; Can detect mutagens in the gaseous phase; Eukaryotic organization of genetic material; Many mutational events can be observed directly; System appears highly sensitive to physical and chemical mutagens.

Weaknesses: Lacks pharmacological relevance; May not be suitable for evaluating many compounds requiring mammalian metabolic activation.

Status of Development: Developmental.

Describe: The *Tradescantia* system was initially developed (and is well-suited) for study of radiation effects. The system is applicable to at least some chemical mutagens and is currently under development for that purpose.

Applications: Air; Water.

Samples: *Pure Chemicals*: Organics, Nitroso derivatives, Polycilchydrocarbons, Nucleic acid bases, and analogs, Hydroxylamines, Hydrasine. *Complex Mixtures*: Ambient - drinking water, air; Industrial - soil.

Duration: 2 to 5 weeks.

Cost: $500 to $700, depending upon the test protocol.

Interpretation: The change in stamen hair cells' color from blue to pink suggests mutagenicity.

Level of Complexity: I.

OHEE Laboratory Involved: HERL-RTP, Environmental Toxicology Division, Biochemistry Branch, Cellular Biology Section; HERL-CIN, Field Studies Division, Toxicological Assessment Branch.

Persons to Contact: *M.D. Waters*, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2693); *S.S. Sandhu*, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2693); *L.J. McCabe*, U.S. EPA, HERL-CIN, 26 W. St. Clair St., Cincinnati, OH 45268, (FTS 684-7211).

Grant/Contract Laboratory Involved and Principal Investigators: Brookhaven National Laboratories, Long Island, New York, L. Shirer; U. of Missouri, Columbia, MO 65201, J.T. O'Connor; Stanford Research Institute, Menlo Park, CA 94205, G. Newell.

Program Office Support: OHEE; OPP.

References: 1) Sparrow, A.H., et al. Mutation Res., 26:265-276, 1974; 2) Underbrink, A.G., et al. In: Chemical Mutagens: Principles and Methods for Their Detection. Vol. 3. A. Hollaender, ed. Plenum Press, NY, 1973. pp. 171-207. 3) McNulty, P.J., et al. Mutation Res., 44:235-246, 1977.

12112 MAIZE WAXY LOCUS ASSAY

Biological Activity Detected: Mutagenicity.

Principle: The assay is based on the change from the ability of the plants to synthesize amylose to the inability to synthesize this compound. Pollen from the treated plants is stained with iodine. Mutated pollen grains are stained purple.

Endpoints: Qualitative: Change in pollen grain color from yellow to purple. Quantitative: Induced mutation frequency is expressed.

Strengths: Test is performed in-vivo representing relevant conditions of exposure; Damage to germ cells is measured.

Weaknesses: Time consuming.

Status of Development: Developmental.

Describe: Few pure compounds or mixtures have been tested in this system. This test system appears promising but needs validation.

Applications: Air; Soil; Multimedia.

Samples: Pure Chemicals: Pesticides. Complex Mixtures: Industrial.

Duration: 8 weeks.

Cost: Unknown.

Interpretation: Change in pollen grain color after staining suggests mutation.

Level of Complexity: 2.

OHEE Laboratory Involved: HERL-RTP, Environmental Toxicology Division, Biochemistry Branch, Cellular Biology Section.

Persons to Contact: M.D. Waters, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2693); S.S. Sandhu, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2693).

Grant/Contract Laboratory Involved and Principal Investigators: U. of Illinois, Urbana, IL 61801, M. Plewa.

Program Office Support: OPP.

References: 1) Plewa, M., and J. Gentile. Maize Cooper. Newsletter, 50:44, 1976. 2) Plewa, M., and J. Gentile. Mutation Res., 38:287-292, 1976.

12113 IN-VIVO CYTOGENETICS IN MICE

Biological Activity Detected: Mutagenicity.

Principle: Chemicals are administered into mice through various routes. After a specific period, treated animals are sacrificed. Bone marrow and spermatogonial cells are analyzed for chromosomal aberrations.

Endpoints: Qualitative: Observation of chromosomal and chromatid breaks. Quantitative: Number of breaks/cell as compared to the control, provides a quantitative assessment of treatment response.

Strengths: In-vivo bioassay provides the benefit of intact pharmokinetics.

Weaknesses: Expensive; Requires a well trained personnel to perform the test and interpret the data.

Status of Development: Validated.

Describe: N/A.

Applications: Multimedia.

Samples: Pure Chemicals: Alkylating agents. Complex Mixtures: Protocol not yet developed.

Duration: 3 weeks.

Cost: $3,000 to $6,500/chemical.

Interpretation: A significant increase in chromosomal aberrations over the control suggests mutation.

Level of Complexity: 5.

OHEE Laboratory Involved: HERL-RTP, Environmental Toxicology Division, Biochemistry Branch, Cellular Biology Section; HERL-CIN, Field Studies Division, Toxicological Assessment Branch.

Persons to Contact: M.D. Waters, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2693); S.S. Sandhu, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2693); L. Claxton, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2942); J.F. Stara, U.S. EPA, HERL-CIN, 26 W. St. Clair St., Cincinnati, OH 45268, (FTS 684-7407).

Grant/Contract Laboratory Involved and Principal Investigators: U. of Texas, Medical Branch, Galveston, TX 77550, M. Legator; Stanford Research Institute, Menlo Park, CA 94205, G. Newell.

Program Office Support: OHEE, OPP.

References: 1) Evans, H.J. In: Chemical Mutagens: Principles and Methods for Their Detection. Vol. 4. A. Hollander, ed. Plenum Press, NY, 1977. 2) Schmid, W. In: Chemical Mutagens: Principles and Methods for Their Detection. Vol. 4. A. Hollander, ed. Plenum Press, NY, 1977. pp. 31-52.

CONTENTS: TEST SYSTEMS

1220 DNA Damage. 73
  1221 *Bacillus subtilis* rec$^-$. 74
  1221 *Escherichia coli*, Pol A$^-$. 75
  1223 Mitotic Recombination and Gene Conversion in *Saccaromyces cerevisiae*. 76
  1224 Unscheduled DNA Synthesis (UDS) 77
  1225 Sister-Chromatid Exchange Formation (SCE) 78
  1226 In-Vivo Assessment of DNA Damage. 79
  1227 Intact Rodent Hepatocytes in Primary Culture 80
  1228 In-Vivo DNA Binding 81
  1229 Chinese Hamster Cells (CHO) Unscheduled DNA Synthesis (UDS) 82
1230 Carcinogenesis. 83

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1221 BACILLUS SUBTILIS REC$^-$

Biological Activity Detected: Toxicity; Primary DNA damage.

Principle: The DNA recombinational repair deficient and proficient strains are streaked out along the intersecting lines. The test chemical is spotted at the intersection. The differential killing between the repair deficient and proficient strains is used as a criteria of DNA damage.

Endpoints: Qualitative: A comparison is made between the zone of growth inhibition for the repair deficient and proficient strains. Quantitative: N/A.

Strengths: Very rapid and versatile bioassay; Inexpensive.

Weaknesses: Requires fairly large amount of test substances for testing; Not suitable for substances which do not diffuse readily in agar.

Status of Development: Validated.

Describe: N/A.

Applications: Multimedia.

Samples: Pure Chemicals: Alkylating agents, Nitroso compounds, Polynuclear aromatics, Nitroso derivatives; Pesticides. Complex Mixtures: Ambient; Industrial; Energy Related; Transportation Related; Other.

Duration: 2 to 3 weeks.

Cost: $200.

Interpretation: Relative sizes of killing zones in repair-proficient and repair-deficient stains indicate primary damage to DNA.

Level of Complexity: I.

OHEE Laboratory Involved: HERL-RTP, Environmental Toxicology Division, Biochemistry Branch.

Persons to Contact: M.D. Waters, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2693); L. Claxton, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2942); S.S. Sandhu, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2693); J.L. Huisingh, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2948).

Grant/Contract Laboratory Involved and Principal Investigators: Stanford Research Institute, Menlo Park, CA 94205, V.F. Simmons.

Program Office Support: OHEE; OPP.

References: 1) Kada, T., K. Tutikawa, and Y. Sadaie. Mutation Res., 16:165-174, 1972.

1222 ESCHERICHIA COLI, Pol A^-.

Biological Activity Detected: Toxicity; Primary DNA damage.

Principle: The assay measures the differential killing between DNA repair proficient Escherichia coli strain (W3110, Pol A^+) and DNA repair deficient strain (P3478, Pol A^-) as affected by environmental toxicants.

Endpoints: Qualitative: Differential killing between DNA repair proficient and deficient strain after treatment with a test-substance. Quantitative: Liquid suspension test provides a quantitative measure of primary DNA damage.

Strengths: The assay is well suited for detecting chemicals causing frame shift mutations; Adequate data base is present on this bioassay; Genetically well-characterized system; Rapid; Inexpensive; Well validated as a test for gene mutation; Works well with in-vitro metabolizing microsome fractions; Can be used as indicator organism in host-mediated assays.

Weaknesses: Reverse mutation assay requiring several strains to permit detection of a broad spectrum of compounds; Requires metabolic activation; Lacks pharmacological relevance, Prokaryotic organization of genetic material.

Status of Development: Validated.

Describe: N/A.

Applications: Multimedia.

Samples: Pure Chemicals: Alkylating agents, Nitroso compounds, Polynuclear aromatics, Nitroso derivatives, pesticides. Complex Mixtures: Ambient; Industrial; Energy Related; Transportation Related; Other.

Duration: 2 to 4 weeks.

Cost: $500.

Interpretation: Relative sizes of killing zones in repair-proficient and repair-deficient stains indicate primary damage to DNA.

Level of Complexity: I.

OHEE Laboratory Involved: HERL-RTP, Environmental Toxicology Division, Biochemistry Branch.

Persons to Contact: M.D. Waters, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2693); L. Claxton, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2942).

Grant/Contract Laboratory Involved and Principal Investigators: Stanford Research Institute, Menlo Park, CA 94205, V.F. Simmons.

Program Office Support: OHEE; OPP.

References: 1) Rosenbranz, H.S., et al. Mutation Res., 41:61-70, 1976. 2) Rosenbranz, H.S. Ann. Res. Microbiol., 27:383-401, 1973. 3) Rosenbranz, H.S. Cancer Res., 33:458-459, 1973.

1223 MITOTIC RECOMBINATION AND GENE CONVERSION IN *SACCHAROMYCES CEREVISIAE*

Biological Activity Detected: Primary DNA damage.

Principle: Recombination of reciprocal type, mitotic recombination, and the non-reciprocal type mitotic gene conversion are used for assessing the DNA damaging potential of environmental chemicals.

Endpoints: Qualitative: Appearance of twin spots and growth in selective medium. Quantitative: The degree of mitotic crossing-over is evaluated by the frequency of twin spot sectors and that of mitotic gene conversion by the differential growth in a selective medium.

Strengths: The diploid cells with eukaryotic chromosomal organization; Rapid; Inexpensive.

Weaknesses: Less versatile due to problems associated with cell wall permeability and with coupling of metabolic activation; Inadequate data base showing the reliability of this assay system.

Status of Development: Being Implemented.

Describe: This test system needs to be validated with a variety of classes of compounds. Further work is needed in understanding the mechanism and significance of mitotic recombination and crossing-over.

Applications: Multimedia.

Samples: Pure Chemicals: Alkylating agents, Nitrosamines, Carbamates, Nucleic acid bases and analogs, Aromatic amines. Complex Mixtures: Protocol not yet developed.

Duration: 2 to 3 weeks.

Cost: $200 to $500.

Interpretation: The appearance of twin spots and growth in a selective medium suggests mutation.

Level of Complexity: I.

OHEE Laboratory Involved: HERL-RTP, Environmental Toxicology Division, Biochemistry Branch.

Persons to Contact: M.D. Waters, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2693); S.S. Sandhu, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2693).

Grant/Contract Laboratory Involved and Principal Investigators: Stanford Research Institute, Menlo Park, CA 94205, V.F. Simmons.

Program Office Support: OHEE; OPP.

References: 1) Zimmermann, F.K. Mutation Res., 31:71-86, 1975.
2) Brusick, D.J., and V.W. Mayer. Envir. Hlth. Prospect., 6:83, 1973.

1224 UNSCHEDULED DNA SYNTHESIS (UDS)

Biological Activity Detected: Primary DNA.

Principle: This assay evaluates the test compounds for their ability to induce unscheduled DNA synthesis (UDS) in human diploid WI38 fibroblasts blocked in the G_1 phase.

Endpoints: Qualitative: Incorporation of ^{3}H thymidine. Quantitative: dpm ^{3}H thymidine per μg or μmole of DNA.

Strengths: DNA repair can be measured in human cells in culture; Similar studies can be performed in animals using peripheral leucocytes; The latter permit comparison between in-vitro and in-vivo exposures to carcinogens or mutagens.

Weaknesses: The precise type of molecular binding between carcinogens and DNA which triggers excision repair is unknown; DNA repair synthesis does not measure residual damage to DNA.

Status of Development: Validated.

Describe: The mechanism of UDS is still not completely understood.

Applications: Multimedia.

Samples: Pure Chemicals: Alkylalois; Alkylating agents, Nitroso compounds, Polynuclear aromatics. Complex Mixtures: Ambient; Industrial; Energy Related; Transportation Related; Other.

Duration: 4 to 6 weeks.

Cost: $350 to $2,000.

Interpretation: The incorporation of labeled nucleotide precursors into the cells arrested in the G_1 phase after treatment is used as a criteria of the ability of the test material to cause primary DNA damage.

Level of Complexity: 2.

OHEE Laboratory Involved: HERL-RTP, Environmental Toxicology Division, Biochemistry Branch.

Persons to Contact: M.D. Waters, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2693).

Grant/Contract Laboratory Involved and Principal Investigators: Stanford Research Institute, Menlo Park, CA 94205, A. Mitchell.

Program Office Support: OHEE; OPP.

References: 1) Stich, H., and S. Laighes. DNA Repair and Chemical Carcinogenesis. Pathobiol. Ann., 3:342-376, 1973. 2) San, R.H., and H.F. Stich. DNA Repair Synthesis of Cultured Human Cells as a Rapid Bioassay for Chemical Carcinogens. Int. J. Cancer, 16: 284-291, 1975. 3) Williams, G.M. Detection of Chemical Carcinogens by Unscheduled DNA Synthesis in Rat Liver Primary Cell Cultures. Cancer Res., 37:1845-1851, 1977. 4) Simmon, V.F., A.D. Mitchell, and T.A. Jorgenson. Evaluation of Selected Pesticides as Chemical Mutagens: In-vitro and In-vivo Studies. Ann. Rep., Envir. Hlth. Effects Series, EPA-600/1-77-028, 1977.

1225 SISTER-CHROMATID EXCHANGE FORMATION (SCE)

Biological Activity Detected: Primary DNA damage.

Principle: SCE involves a reciprocal exchange between sister-chromatids which does not result in a change in the overall chromosonal morphology. SCE may be observed as darkly staining and lightly staining chromatids after growth in BUdR for two successive cell generations and subsequent-staining with fluorochrome dyes.

Endpoints: Qualitative: Observation of sister-chromatid exchanges of metaphase. Quantitative: SCE/cell are expressed.

Strengths: Rapid; Relatively economical; Very sensitive; Can be tested in-vivo or in-vitro.

Weaknesses: Mechanism and significance of SCE is not understood; No clear relationship between SCE and chromosomal breaks has been established.

Status of Development: Being implemented.

Describe: The validation of this system is near completion in several laboratories.

Applications: Multimedia.

Samples: Pure Chemicals: Alkylating agents, Mycotoxins, Halogenated hydrocarbons, Ureas and Thioureas, Nitro derivatives. Complex Mixtures: Protocol not yet developed.

Duration: 2 to 4 weeks.

Cost: $1,000 to $1,200.

Interpretation: Test agents inducing signigicant numbers of sister-chromatid exchanges may be regarded as potential DNA-damaging agents for animals and man.

Level of Complexity: 3.

OHEE Laboratory Involved: HERL-RTP, Environmental Toxicology Division, Biochemistry Branch; ERL-NAR, Toxicology Branch, Genetic Toxicology Team.

Persons to Contact: M.D. Waters, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2693); S.S. Sandhu, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2693); A.R. Malcolm, U.S. EPA, ERL-NAR, South Ferry Rd., Narragansett, RI 02882, (FTS 838-4843); G.G. Pesch, U.S. EPA, ERL-NAR, South Ferry Rd., Narragansett, RI 02882, (FTS 838-4843).

Grant/Contract Laboratory Involved and Principal Investigators: Stanford Research Institute, Menlo Park, CA 94205, V.F. Simmons.

Program Office Support: OHEE; OPP.

References: 1) Perry, P., and H.J. Evans. Nature, 258;121-125, 1975. 2) Latt, S.A. Proc. Natl. Acad. Sci., 70:3395-3399, 1973. 3) Popescur, N.C., et al. F. Natl. Cancer Inst., 59:289-293, 1977.

1226 IN-VIVO ASSESSMENT OF DNA DAMAGE

Biological Activity Detected: Primary DNA damage.
Principle: Detection of in-vivo DNA repair activity that is stimulated by chemical carcinogens.
Endpoints: Qualitative: Initial endpoint-measurement of DNA molecular weight change. Quantitative: Molecular weight distribution of cleaved DNA strands.
Strengths: Detects DNA damage in-vivo; Assay is done on biopsy material; Non-destructive, animal sampled can later be scored for tumors to validate assay.
Weaknesses: Not yet apparent; Mainly technical.
Status of Development: Developmental.
Describe: Still in early stages of development.
Applications: Multimedia.
Samples: Pure Chemicals: Organic compounds, Inorganics, Heavy metals. Complex Mixtures: Ambient - water; Industrial; Energy Related; Transportation Related; Other.
Duration: Variable, but not to exceed 3 months once validated.
Cost: Not yet determined.
Interpretation: Mutagenic and carcinogenic agents act through damage of DNA. This method will quantitate DNA damage.
Level of Complexity: 3.
OHEE Laboratory Involved: HERL-CIN, Laboratory Studies Division, Toxicological Assessment Branch.
Persons to Contact: R.J. Bull, U.S. EPA, HERL-CIN, 26 W. St. Clair St., Cincinnati, OH 45268, (FTS 684-7213).
Grant/Contract Laboratory Involved and Principal Investigators: Ohio State University, Chemical Biomedical Environmental Research Group, Columbus, OH 43210, R.W. Hart, (FTS 940-9375).
Program Office Support: OHEE; OWHM.
References: 1) Brash, et al. N.Y. Acad. J.C., 1977. 2) Brash and Hart, R.W. Envir. Hlth. Perspect., 1978. In press.

1227 INTACT RODENT HEPATOCYTES IN PRIMARY CULTURE

Biological Activity Detected: Toxicity; Presumptive mutagenicity; Oncogenicity; DNA damage.

Principle: Detects interaction of chemical agents which result in DNA damage. This interaction is detected as unscheduled DNA synthesis using radiotracer, centrifugal and autoradiographic techniques.

Endpoints: Qualitative: A positive response suggests potential to act as a mutagen or carcinogen. Quantitative: Can quantify the number of grains/nucleus which increases with increased dose.

Strengths: Rapid; Economical; The entire genome is the target; Maintains several functions of tissue of origin, thereby activating many different chemicals; Not limited to direct acting compounds.

Weaknesses: Represents only a single target organ; System lacks validation; May not detect promotors or co-carcinogens.

Status of Development: Developmental.

Describe: This system is now in the process of validation in several laboratories. It has not yet been applied to unknown compounds.

Applications: Multimedia.

Samples: Pure Chemicals: Aromatic amines, Polycyclics, Alkylators, Hormones. Complex Mixtures: Not yet determined.

Duration: 2 days to 2 weeks, depending on technique.

Cost: $500 to $2,000.

Interpretation: This test is considered a reliable screen for prioritization of chemical testing in more complex in-vivo systems. It suggests that the chemical reaches the genome and requires further analysis.

Level of Complexity: 3 to 4.

OHEE Laboratory Involved: National Center for Toxicological Research, Division of Mutagenesis Research, Somatic Cell Section; HERL-RTP, Environmental Toxicology Division, Biochemistry Branch, Cellular Biology Section.

Persons to Contact: D.A. Casciano, National Center for Toxicological Research, Jefferson, AR 72079, (FTS 740-4573); J.L. Huisingh, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2948).

Grant/Contract Laboratory Involved and Principal Investigators: American Health Foundation, Naylor Dana Institute for Disease Prevention, Hammond House Road, Valhalla, NY 10595, G.M. Williams.

Program Office Support: OHEE.

References: 1) Kitagawa, T., et al. Cancer Res., 35:3682-3692, 1975. 2) Michalopoulos, G., et al. Life Sciences, 18:1139-1144, 1976. 3) Williams, G.M. Cancer Letters, 1:231-236, 1976. 4) San, R.H.C., and H.F. Stich. Intl. J. Cancer, 7:65-74, 1971.

1228 IN-VIVO DNA BINDING

Biological Activity Detected: Binding of chemical to DNA.

Principle: This test system attempts to correlate binding with DNA repair and tumorigenicity. Various rodent stains will be treated with the chemical carcinogen DNA from presumptive target tissue analyzed for DNA-carcinogen adducts by radiometric and/or fluorometric techniques, measuring the rate of excision of the bound chemical.

Endpoints: Qualitative: Adducts will be determined by chromatographic procedures. Quantitative: Can quantify amount of carcinogen bound/unit DNA (μ mole carcinogen/mole DNA-phosphate).

Strengths: A direct measurement of the extent of carcinogen interaction with DNA; Allows measurement of total DNA damage.

Weaknesses: A considerable portion of the DNA-carcinogen adducts may have little or no biological relevance.

Status of Development: Developmental.

Describe: Initial experiments are being carried out to provide background for development of in-vitro DNA repair assay.

Applications: Multimedia.

Samples: Pure Chemicals: Organic-metallic compounds, Organics. Complex Mixtures: N/A.

Duration: 2 weeks.

Cost: Not yet determined.

Interpretation: This test measures the direct interaction of the test compound with genetic material as an indication of carcinogenic and mutagenic activity.

Level of Complexity: 3.

OHEE Laboratory Involved: HERL-CIN, Laboratory Studies Division, Toxicological Assessment Branch.

Persons to Contact: B. Daniel, U.S. EPA, HERL-CIN, 26 W. St. Clair St., Cincinnati, OH 45268, (FTS 684-7482).

Grant/Contract Laboratory Involved and Principal Investigators: N/A.

Program Office Support: OHEE; OWHM.

References: Not yet available.

1229 CHINESE HAMSTER CELLS (CHO) UNSCHEDULED DNA SYNTHESIS (UDS)

Biological Activity Detected: DNA Repair.
Principle: Repair of induced damage to DNA is detected as unscheduled DNA synthesis via incorporation of ^{3}H thymidine.
Endpoints: Qualitative: Unscheduled DNA synthesis is measured and compared to controls. Quantitative: Amount of unscheduled DNA synthesis per cell may be determined.
Strengths: Rapid; Relatively low cost; DNA repair is probably a more sensitive detector of DNA damage than are chromosomal aberrations.
Weaknesses: The in-vitro cell assay lacks pharmacological relevance; Requires metabolic activation.
Status of Development: Being implemented.
Describe: The test is presently being applied to standard compounds and some selected unknowns. It is still being refined and modified.
Applications: Water; Multimedia.
Samples: Pure Chemicals: Organics (EMS), Heavy metals, UV radiation. Complex Mixtures: Energy Related - JP-5 jet fuel.
Duration: 2 to 3 weeks.
Cost: $1,000 to $1,200/compound.
Interpretation: Agents inducing significant unscheduled DNA synthesis represent potential carcinogens/mutagens for animals and man.
Level of Complexity: 2.
OHEE Laboratory Involved: ERL-NAR, Toxicology Branch, Genetic Toxicology Team.
Persons to Contact: E. Jackim, U.S. EPA, ERL-NAR, South Ferry Rd., Narragansett, RI 02882, (FTS 838-4843 X229, X310); A.R. Malcolm, U.S. EPA, ERL-NAR, South Ferry Rd., Narragansett, RI 02882, (FTS 838-4843 X238, X247).
Grant/Contract Laboratory Involved and Principal Investigators: N/A.
Program Office Support: OHEE.
References: San, R.H.C., and H.F. Stitch. Int. J. Cancer, 16:284-291, 1975. 2) Martin, C.N., et al. Cancer Letters, 2:355-360, 1977. 3) Trosko, J.E., and J.D. Yager. Exp. Cell Res., 88:47-55, 1974.

CONTENTS: TEST SYSTEMS

1230 Carcinogenesis. 83

1231 C3H10T1/2CL8 Mouse Embryo Fibroblast Oncogenic Transformation with Exogenous Metabolic Activation. 84

1232 C3H10T1/2CL8 Mouse Embryo Fibroblast Oncogenic Transformation. 85

1233 BHK-21 Mammalian Cell Oncogenic Transformation. 86

1234 Syrian Hamster Embryo Oncogenic Transformation (Focus Assay). 87

1235 BALB 3T3 Oncogenic Transformation and Mutagenesis with Exogenous Metabolic Activation. 88

1236 BALB 3T3 Oncogenic Transformation 89

1237 Pulmonary Adenoma Bioassay in Mice. 90

1240 Marine Applications 91

1231 C3H10T1/2CL8 MOUSE EMBRYO FIBROBLAST ONCOGENIC TRANSFORMATION WITH EXOGENOUS METABOLIC ACTIVATION

Biological Activity Detected: Presumptive oncogenicity.

Principle: Normal cells in log phase are treated with the test agent. Four weeks after the cells have attained confluence they are scored for morphologically transformed foci (clones of cells). These transformed cells will give rise to tumors when injected into immunosupressed syngeneic animals.

Endpoints: Qualitative: Appearance of morphologically transformed foci. Quantitative: Simultaneous cytotoxicity experiments are performed to obtain the lethal toxicity of the agent and transformation is then adjusted for that toxicity.

Strengths: This system is easy to score; Has an extremely low background of spontaneous transformation; Is particularly sensitive to PAH and their derivatives; Has metabolic activation capability; Can be used to detect tumor promotors and initiators.

Weaknesses: These mouse embryo cells are aneuploid; Requires 6 weeks to complete the experiment; Seems to be somewhat refractory to the carcinogenic effects of alkylating agents, aflatoxin B_1 and some aromatic amines.

Status of Development: Developmental.

Describe: The C3H10T1/2 system is being modified by the addition of exogenous metabolic activation capability, giving, therefore, increased sensitivity towards a broad range of chemical carcinogens.

Applications: Air.

Samples: Pure Chemicals: Polycyclic aromatic hydrocarbons.

Complex Mixtures: N/A.

Duration: 5 to 6 weeks.

Cost/sample or chemical: $5,000 to $7,000.

Interpretation: The appearance of morphologically altered clones of cells indicates oncogenic transformation.

Level of Complexity: 4.

OHEE Laboratory Involved: HERL-RTP, Environmental Toxicology Division, Biochemistry Branch, Metabolic Effects Section.

Persons to Contact: S. Nesnow, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2693).

Grant/Contract Laboratory Involved and Principal Investigators: Microbiological Associates, Bethesda, MD 20014, R. Kouri, L. Schectman.

Program Office Support: OEMI.

References: 1) Reznikoff, et al. Cancer Res., 33:3231-3249, 1973. 2) Nesnow, et al. Cancer Res., 36:1801-1808, 1976. 3) Mondal, et al. Cancer Res., 36:2254-2260, 1976. 4) Bertram. Cancer Res., 37:514-523, 1977. 5) Benedict, et al. Cancer Res., 37:2202-2208, 1977.

1232 C3H10T1/2CL8 MOUSE EMBRYO FIBROBLAST ONCOGENIC TRANSFORMATION

Biological Activity Detected: Presumptive oncogenicity.

Principle: Normal cells in log phase are treated with the test agent. Four weeks after the cells have attained confluence they are scored for morphologically transformed foci (clones of cells). These transformed cells will give rise to tumors when injected into immunosupressed syngeneic animals.

Endpoints: Qualitative: Appearance of morphologically transformed foci. Quantitative: Simultaneous cytotoxicity experiments are performed to obtain the lethal toxicity of the agent and the transformation is then adjusted for that toxicity.

Strengths: This system is easy to score; Has an extremely low background of spontaneous transformation; Is particularly sensitive to PAH and their derivatives; Has metabolic activation capability; Can be used to detect tumor promotors and initiators.

Weaknesses: These mouse embryo cells are aneuploid; Requires 6 weeks to complete the experiment; Seems to be somewhat refractory to the carcinogenic effects of alkylating agents, aflatoxin B_1, and some aromatic amines.

Status of Development: Being implemented.

Describe: The C3H10T1/2 system is being modified so that its metabolic activation capability is increased, giving, therefore, increased sensitivity towards a broad range of chemical carcinogens.

Applications: Multimedia.

Samples: Pure Chemicals: Polycyclic aromatic hydrocarbons, Aromatic azo dyes, aromatic amines, pesticides. Complex Mixtures: Model particulates.

Duration: 5 to 6 weeks.

Cost/sample: $5,700 to $7,000.

Interpretation: The appearance of morphologically altered clones indicates oncogenic transformation.

Level of Complexity: 4.

OHEE Laboratory Involved: HERL-RTP, Environmental Toxicology Division, Biochemistry Branch, Metabolic Effects Section.

Persons to Contact: S. Nesnow, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2693).

Grant/Contract Laboratory Involved and Principal Investigators: In-house.

Program Office Support: OHEE; OPP.

References: 1) Reznikoff, et al. Cancer Res., 33:3231-3249, 1973. 2) Nesnow, et al. Cancer Res., 36:1801-1808, 1976. 3) Mondal, et al. Cancer Res., 36:2254-2260, 1976. 4) Bertram. Cancer Res., 37:514-523, 1977. 5) Benedict, et al. Cancer Res., 37:2202-2208, 1977.

1233 BHK-21 MAMMALIAN CELL ONCOGENIC TRANSFORMATION

Biological Activity Detected: Presumptive oncogenicity.

Principle: Normal baby hamster kidney fibroblasts do not grow in soft agar. After treatment with carcinogens these cells do grow in soft agar and will give tumors when injected into syngeneic animals.

Endpoints: Qualitative: Cells which grow in soft agar are considered transformed. Quantitative: Colony formation is scored.

Strengths: Not yet known.

Weaknesses: Not yet known.

Status of Development: Developmental.

Describe: Testing of unconcentrated and concentrated wastewater samples.

Applications: Water.

Samples: Pure Chemicals: PAH. Complex Mixtures: Wastewaters.

Duration: 3 weeks.

Cost/sample or chemical: Not yet known.

Interpretation: A positive result suggests a possible carcinogenic material.

Level of Complexity: 3.

OHEE Laboratory Involved: HERL-CIN, Field Studies Division, Toxicological Assessment Branch.

Persons to Contact: H. Pahren, U.S. EPA, HERL-CIN, 26 W. St. Clair St., Cincinnati, OH 45268, (FTS 684-7217).

Grant/Contract Laboratory Involved and Principal Investigators: Syracuse Research Corporation, Merrill Lane, Syracuse, NY 13210, J. Saxena.

Program Office Support: OHEE.

References: 1) Bouck, N., and G. diMayorca. Nature, 264:722-727, 1976.

1234 SYRIAN HAMSTER EMBRYO ONCOGENIC TRANSFORMATION (FOCUS ASSAY)

Biological Activity Detected: Presumptive oncogenicity.

Principle: Freshly isolated cells from hamster fetuses are seeded into dishes, passaged twice, and then treated for two consecutive three drug treatments with the test agents. Toxicity is scored 1 to 2 days after the experiment is begun and the transformation (appearance of morphologically transformed foci) is scored 10 days after toxicity.

Endpoints: Qualitative: Appearance of morphologically transformed foci. Quantitative: Simultaneous cytotoxicity experiments are performed to obtain the lethal toxicity of the agent and the transformation is then adjusted for that toxicity.

Strengths: These cells have high metabolic activation capability; Are diploid; Respond to a wide variety of different chemical agents; Few false positives are known.

Weaknesses: Variability within the assay due to variations in obtaining and preparing viable primary cell cultures; Observable spontaneous transformation background; Difficulty in scoring.

Status of Development: Being implemented.

Describe: The syrian hamster embryo biassay is being evaluated for use in the evaluation of particulate samples.

Applications: Air.

Samples: Pure Chemicals: Polycyclic aromatic hydrocarbons.
Complex Mixtures: Model particulates.

Duration: 1 month.

Cost/assay: $4,000 to $5,000.

Interpretation: The appearance of morphologically altered clones of cells indicates oncogenic transformation.

Level of Complexity: 4.

OHEE Laboratory Involved: HERL-RTP, Environmental Toxicology Division, Biochemistry Branch, Metabolic Effects Section.

Persons to Contact: S. Nesnow, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2693); M.D. Waters, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2693).

Grant/Contract Laboratory Involved and Principal Investigators: In-house.

Program Office Support: OHEE.

References: 1) Casto, B.C., N. Janosko, and J.A. DiPaolo. Cancer Res., 37:3508-3515, 1977.

1235 BALB 3T3 ONCOGENIC TRANSFORMATION AND MUTAGENESIS WITH EXOGENOUS METABOLIC ACTIVATION

Biological Activity Detected: Mutagenicity; Presumptive oncogenicity.

Principle: Mammalian cell clones of BALB 3T3 clone A13 undergo malignant transformation upon treatment with known carcinogens. Extent of transformation is expressed in focus formation and altered morphology, increased saturation density and enhanced plating efficiency in soft agar. Simultaneously these cells also undergo a permanent genetic change at the ouabain locus. Tumor induction is observed in recipient animals by reinjecting transformed cells.

Endpoints: Qualitative: Appearance of morphological transformed foci for transformation and clones of cells which grow in ouabain supplanted media. Quantitative: Number of observed foci adjusted for the cytotoxic effect of the agent.

Strengths: BALB 3T3 cells are mouse embryo fibroblasts which have the capability to activate 3-methylcholanthrene to metabolites which transform these cells; Direct acting alkylating agents such as MNNG are also effective transforming agents.

Weaknesses: These cells are not transformed by the carcinogens benzo[a]pyrene or 6-aminochrysene and possibly other potent carcinogens unless exogeneous metabolic activation is provided.

Status of Development: Developmental.

Describe: The addition of rat liver preprarations to activate carcinogens and mutagens to make this assay system more sensitive is currently underway.

Applications: Air.

Samples: Pure Chemicals: PAH, Aromatic amines. Complex Mixtures: Transportation Related.

Duration: 5 to 6 weeks.

Cost/sample or chemical: $5,000 to $7,000

Interpretation: Morphologically altered clones of cells indicate oncogenic transformation. Cells growing in the presence of ouabain are indicative of a mutagenic change.

Level of Complexity: 4.

OHEE Laboratory Involved: HERL-RTP, Environmental Toxicology Division, Biochemistry Branch, Metabolic Effects Section.

Persons to Contact: S. Nesnow, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2693).

Grant/Contract Laboratory Involved and Principal Investigators: Microbiological Associates, Bethesda, MD 20014, L. Schectman, R. Kouri.

Program Office Support: OEMI.

References: 1) Kakunaga, T. A Quantitative Assay for Malignant Transformation by Chemical Carcinogens Using Clone from BALB 3T3. Int. J. Cancer, 12:463, 1973.

1236 BALB 3T3 ONCOGENIC TRANSFORMATION

Biological Activity Detected: Presumptive oncogenicity.

Principle: Mammalian cell clones of BALB 3T3 clone A13 undergo malignant transformation with known carcinogens. Extent of transformation is expressed in focus formation and altered morphology, increased saturation density, and enhanced plating efficiency in soft agar. Tumor induction is observed in recipient animals by reinjecting transformed cells.

Endpoints: Qualitative: Appearance of morphological transformed foci. Quantitative: Number of observed foci adjusted for the cytotoxic effect of the agent.

Strengths: BALB 3T3 cells are mouse embryo fibroblasts which have the capability to activate 3-methylcholanthrene to metabolites which transform these cells; Direct acting alkylating agents such as MNNG are also effective transforming agents.

Weaknesses: These cells are not transformed by the carcinogens benzo(a)pyrene or 6-aminochrysene and possibly other potent carcinogens.

Status of Development: Being implemented.

Describe: Currently BALB/T3 cell culture is used for routine testing, but other cell lines (e.g. epithelial) are being investigated in an effort to increase sensitivity.

Applications: Water.

Samples: Pure Chemicals: Organics. Complex Mixtures: Ambient - drinking water; Other - advanced waste treatment, concentrate effluent.

Duration: 5 to 6 weeks.

Cost/sample or chemical: $5,000 to $7,000.

Interpretation: A positive result indicates possible carcinogenesis.

Level of Complexity: 2.

OHEE Laboratory Involved: HERL-CIN, Field Studies Division, Toxicological Assessment Branch; HERL-CIN, Laboratory Studies Division, Toxicological Assessment Branch.

Persons to Contact: J.P. Bercz, U.S. EPA, HERL-CIN, 26 W. St. Clair St., Cincinnati, OH 45268, (FTS 684-7432); N.E. Kowal, U.S. EPA, HERL-CIN, 26 W. St. Clair St., Cincinnati, OH 45268, (FTS 684-7477); R.J. Bull, U.S. EPA, HERL-CIN, 26 W. St. Clair St., Cincinnati, OH 45268, (FTS 684-7213).

Grant/Contract Laboratory Involved and Principal Investigators: U. of Cincinnati, Medical Center, Cincinnati, OH 45221, J.C. Loper and D. Lang; Gulf South Research Institute, P.O. Box 26518, New Orleans, LA 70186, N. Gruener.

Program Office Support: OHEE.

References: 1) Kakunaga, T. A Quantitative Assay for Malignant Transformation by Chemical Carcinogens Using Clone From BALB 3T3. Int. J. Cancer, 12:463, 1973.

1237 PULMONARY ADENOMA BIOASSAY IN MICE

Biological Activity Detected: Presumptive oncogenicity.

Principle: After 13 weeks no untreated strain A mice develop lung tumors; however, after 1 year 100% develop lung tumors. When treated with a carcinogen, lung tumors start developing within 13 weeks.

Endpoints: Qualitative: Tumor formation. Quantitative: Average number of tumors/mouse lung in the treated animals as compared to the positive controls (urethane) and untreated controls.

Strengths: Relatively rapid in-vivo carcinogenesis bioassay.

Weaknesses: The adenoma (pulmonary) has no counterpart in human neoplastic pathology.

Status of Development: Being implemented.

Describe: The system has been developed and used in other laboratories, see references.

Applications: Multimedia.

Samples: Pure Chemicals: Metalic compounds, Food additives, chemotherapeutic agents. Complex Mixtures: Industrial; Transportation Related - diesel particulate.

Duration: 13 to 30 weeks.

Cost/sample or chemical: Not yet known.

Interpretation: The formation of visually observable lung nodules indicates oncogenicity of the test substance.

Level of Complexity: 4.

OHEE Laboratory Involved: HERL-CIN, Laboratory Studies Division, Functional Pathology Branch.

Persons to Contact: J. Orthoefer, U.S. EPA, HERL-CIN, 26 W. St. Clair St., Cincinnati, OH 45268, (FTS 684-7434).

Grant/Contract Laboratory Involved and Principal Investigators: N/A.

Program Office Support: OHEE.

References: 1) Shimkin and Stoner. Lung Tumors in Mice: Application to Carcinogenesis Bioassay. Adv. in Cancer Res., 21:1-58, 1975. 2) Stoner, et al. Test for Carcinogenicity of Metallic Compounds by the Pulmonary Tumor Response in Strain A Mice. Cancer Res., 36:1744-1747, May 1976.

CONTENTS: TEST SYSTEMS

1240 Marine Applications 91

  1241 Tumor Induction in Massive Crustaceans, Molluscs, and Teliost Fish. 92

  1242 MFO Induction as an Indicator of Toxicity Exposure. 93

  1243 Limb Regeneration System. 94

  1244 Isogenic Fish 95

  1245 Integrated System: Development of Mutagen/Carcinogen Activation, Concentration, Separation, and Weathering Systems. 96

  1246 Biphenyl Hydroxylase. 97

1300 Perinatal Toxicology. 99

1241 TUMOR INDUCTION IN MASSIVE CRUSTACEANS, MOLLUSCS, AND TELIOST FISH

Biological Activity Detected: Presumptive oncogenicity.
Principle: Animals are exposed to known carcinogens under laboratory conditions and histopathology is performed. Also, feral animals are surveyed for histopathological abnormalities and correlations are established with tissue residues and water concentrations.
Endpoints: Qualitative: Not supplied. Quantitative: Correlation of exposure/response.
Strengths: May have value as sentinel system for water quality and as a model system.
Weaknesses: Field correlation requires laboratory validation.
Status of Development: Being implemented.
Describe: Not supplied.
Applications: Water.
Samples: Pure Chemicals: PNA's. Complex Mixtures: Ambient - water; Industrial - water.
Duration: Not supplied.
Cost: Not supplied.
Interpretation: Not supplied.
Level of Complexity: Not supplied.
OHEE Laboratory Involved: ERL-GB.
Persons to Contact: J. Couch, U.S. EPA, ERL-GB, Sabine Island, Gulf Breeze, FL 32561, (FTS 686-9011).
Grant/Contract Laboratory Involved and Principal Investigators: U. of Oregon, Eugene, OR 97403, M. Mix; U. of Southern Mississippi, Hattiesburg, MI 39401, B.J. Martin.
Program Office Support: OEMI; OHEE.
References: Not supplied.

1242 MFO INDUCTION AS AN INDICATOR OF TOXICITY EXPOSURE

Biological Activity Detected: Toxicity; Mutagenicity; Presumptive oncogenicity.
Principle: Fetal and laboratory reared animals are exposed to ambient environmental conditions as sentinal organisms.
Endpoints: Qualitative: Not supplied. Quantitative: Degree of induction of MFO.
Strengths: Pre-pre screen method for water quality; Provides guidance for chemical analysis programs.
Weaknesses: Nonspecific.
Status of Development: Developmental.
Describe: Not supplied.
Applications: Water.
Samples: Pure Chemicals: PNA's; Complex Mixtures: Ambient - estuarine/marine.
Duration: Not supplied.
Cost: Not supplied.
Interpretation: Positive test indicates that the test animal has recently been exposed to inducer(s) of MFO systems.
Level of Complexity: Not supplied.
OHEE Laboratory Involved: ERL-GB.
Persons to Contact: N. Richards, U.S. EPA, ERL-GB, Sabine Island, Gulf Breeze, FL 32561, (FTS 686-9011); P. Schoor, U.S. EPA, ERL-GB, Sabine Island, Gulf Breeze, FL 32561, (FTS 686-9011).
Grant/Contract Laboratory Involved and Principal Investigators: U. of West Florida, Pensacola, FL 32504, R. Rao.
Program Office Support: OHEE.
References: Not supplied.

1243 LIMB REGENERATION SYSTEM

Biological Activity Detected: Differentiation/teratology.

Principle: Crustacean limbs are removed at predetermined breakpoints and regenerated by a precise sequence of biochemical events.

Endpoints: Qualitative: Interference with limb regeneration - determined by gross morphology and histopathology. Quantitative: Not supplied.

Strengths: Appropriate for marine samples.

Weaknesses: Difficult to extrapolate to humans.

Status of Development: Being implemented.

Describe: Not supplied.

Applications: Water.

Samples: Pure Chemicals: PCP, Colchicine, PNA's. Complex Mixtures: Ambient - estuarine/marine; Energy Related - drilling fluids.

Duration: Not supplied.

Cost: Not supplied.

Interpretation: Pre-screen for teratogens.

Level of Complexity: Not supplied.

OHEE Laboratory Involved: ERL-GB.

Persons to Contact: N. Richards, U.S. EPA, ERL-GB, Sabine Island, Gulf Breeze, FL 32561, (FTS 686-9011).

Grant/Contract Laboratory Involved and Principal Investigators: U. of West Florida, Pensacola, FL 32504, R. Rao.

Program Office Support: OEMI.

References: Not supplied.

1244 ISOGENIC FISH

Biological Activity Detected: Presumptive oncogenicity; Teratology.
Principle: Isogenic fish provide uniform progeny with predictable life stages, and an opportunity to genetically engineer genotypes for susceptability to carcinogens.
Endpoints: Qualitative: Not supplied. Quantitative: Interference with development; Melanoma/melanin proliferation.
Strengths: In-vivo; Rapid.
Weaknesses: Toxic substances may kill test animal before oncogenic/carcinogenic response is elicited; Substances may not be permeable to eggs.
Status of Development: Developmental.
Describe: Not supplied.
Applications: Water.
Samples: Pure Chemicals: PNA's, Nitrosamines, Aflotoxin, etc.
Complex Mixtures: Not supplied.
Duration: 1 month.
Cost: Not yet determined.
Interpretation: Test results indicate presumptive teratogen, presumptive carcinogen, or toxic substance.
Level of Complexity: Not supplied.
OHEE Laboratory Involved: ERL-GB.
Persons to Contact: N. Richards, U.S. EPA, ERL-GB, Sabine Island, Gulf Breeze, FL 32561, (FTS 686-9011).
Grant/Contract Laboratory Involved and Principal Investigators: U. of North Carolina, Chapel Hill, NC 27514, D. Humm.
Program Office Support: OEMI.
References: Not supplied.

1245 INTEGRATED SYSTEM: DEVELOPMENT OF MUTAGEN/CARCINOGEN ACTIVATION, CONCENTRATION, SEPARATION, AND WEATHERING SYSTEMS

Biological Activity Detected: Toxicity; Mutagenicity.
Prinicple: The above procedures are being developed for use with quick screen tests. Biological, physical, and chemical methods are being used to concentrate, separate, and activate compounds which interfere with testing.
Endpoints: Qualitative: Sample concentrated, freed of interfering substances, activated, prepared for testing. Quantitative: Not supplied.
Strengths: Separation of toxic components from complex mixtures will allow detection; Concentration of dilute genotoxics will allow their detection; Weathering may assist in prediction of the environmental fate; Marine activation systems aid in predicting biological fate and food web relationships.
Weaknesses: Extensive exploratory research is required to validate the methods.
Status of Development: Developmental.
Describe: Not supplied.
Applications: Water; Food.
Samples: Pure Chemicals: PNA's; Complex Mixtures: Ambient - estuarine/marine H_2O; Energy Related - shale; Other - tissue residues.
Duration: Not supplied.
Cost: Not supplied.
Interpretation: These methods may be useful for all in-vitro and in-vivo methods to expand their application to complex samples.
Level of Complexity: Not supplied.
OHEE Laboratory Involved: ERL-GB.
Persons to Contact: N. Richards, U.S. EPA, ERL-GB, Sabine Island, Gulf Breeze, FL 32561, (FTS 686-9011).
Grant/Contract Laboratory Involved and Principal Investigators: Gulf South Research Institute, E. Kline; U. of West Florida, Pensacola, FL 32504, R. Rao.
Program Office Support: OEMI; OHEE.
References: Not supplied.

1246 BIPHENYL HYDROXYLASE

Biological Activity Detected: Presumptive oncogenicity.
Principle: Pre-pre screen for carcogenicity depends on correlation of interference with hydroxylation.
Endpoints: Qualitative: Not supplied. Quantitative: Interference with enzyme reaction.
Strengths: Extremely rapid; Inexpensive.
Weaknesses: Requires extensive development, modification and validation; Current data based on correlation only; Mechanism unknown.
Status of Development: Developmental.
Describe: Requires extensive development, modification, and validation.
Applications: Water; Multimedia.
Samples: Pure Chemicals: Multiple classes being screened.
Complex Mixtures: Not supplied.
Duration: Not supplied.
Cost: Not supplied.
Interpretation: After validation and development, the test may be useful as a rapid, inexpensive pre-pre screen.
Level of Complexity: 0.
OHEE Laboratory Involved: ERL-GB.
Persons to Contact: N. Richards, U.S. EPA, ERL-GB, Sabine Island, Gulf Breeze, FL 32561, (FTS 686-9011).
Grant/Contract Laboratory Involved and Principal Investigators: Denver Research Institute, Denver, CO 80210, J. Schmidt-Coderis.
Program Office Support: OEMI.
References: Not supplied.

CONTENTS: TEST SYSTEMS

1300 Perinatal Toxicology. 99
    1301 Mammalian Teratology. 100
    1302 Perinatal Toxicology. 101
    1303 Fetal Toxicity in Rats, Mice, Guinea Pigs/Hamsters. 102
    1304 Developmental Toxicity in Neonatal Rats 103
    1305 Teratology In-Vivo/In-Vitro 104
    1306 Direct Spectral Measurement of the Biochemical Development of the Nervous System. 105
2000 Short-Term Tests for Ecological Effects 107
2100 Freshwater and Marine Algae 107

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1301 MAMMALIAN TERATOLOGY

Biological Activity Detected: Teratology.
Principle: Determine the potential of the compound to produce anatomical malformations.
Endpoints: Qualitative: N/A. Quantitative: Number and type of malformations.
Strengths: Biologically active compounds are readily detected; Same techniques used for most species.
Weaknesses: Only gross anatomical malformations are noted.
Status of Development: Validated.
Describe: Protocols and methods well established. Compounds are being tested on a routine basis.
Applications: Multimedia.
Samples: Pure Chemicals: All classes. Complex Mixtures: Ambient; Industrial, Energy Related; Transportation Related; Other.
Duration: 6 weeks for small rodents, start to report. Additional gestational time needed for larger animals.
Cost: $6,000/specie/compound.
Interpretation: The teratogenic potential of compounds can be established.
Level of Complexity: 3.
OHEE Laboratory Involved: HERL-RTP, Environmental Toxicology Division, Toxic Effects Branch, Perinatal Toxicology Section.
Persons to Contact: K.D. Courtney, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2370).
Grant/Contract Laboratory Involved and Principal Investigators: N/A.
Program Office Support: OPP.
References: 1) OPP Guidelines for Teratology.

1302 PERINATAL TOXICOLOGY

Biological Activity Detected: Postnatal development.

Principle: Establish biochemical markers to determine normalacy of postnatal development. Isozyme profiles of lactic dehydrogenase (LDH) and creative phosphokinase (CPK) show definite developmental patterns from day 7 to 14 in the postnatal mouse.

Endpoints: Qualitative: N/A. Quantitative: LDH and CPK total activities and isozyme profiles are determined in postnatal mice after prenatal and/or actational exposure. Results are entered into computer by key punch cards and analyzed by established program.

Strengths: Establish a measurable parameter of postnatal development that can be quantified; Determining the cardiac isozymes permits correlation with clinical human data; Comparative species studies use same techniques and interpretations.

Weaknesses: Must wait for animals to be born.

Status of Development: Validated.

Describe: The postnatal isozyme profiles of LDH and CPK have been established and treated animals are being evaluated.

Applications: Multimedia.

Samples: Pure Chemicals: All classes. Complex Mixtures: Ambient; Industrial; Energy Related; Transportation Related; Other.

Duration: Gestation plus 4 weeks, start to report.

Cost: $3,000/compound.

Interpretation: Postnatal development is evaluated with the same techniques that are used in human medicine so that results are directly comparable.

Level of Complexity: 3.

OHEE Laboratory Involved: HERL-RTP, Environmental Toxicology Division, Toxic Effects Branch, Perinatal Toxicology Section.

Persons to Contact: K.D. Courtney, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2370).

Grant/Contract Laboratory Involved and Principal Investigators: N/A.

Program Office Support: OPP.

References: Not yet available.

1303 FETAL TOXICITY IN RATS, MICE, GUINEA PIGS/HAMSTERS

Biological Activity Detected: Fetal toxicity including teratogenicity.

Principle: Pregnant females are treated with chemical (or other agent) during the period of major organogenesis of their litters. The animals are sacrificed before term and the fetuses subsequently examined for signs of toxicity including visual and skeletal defects.

Endpoints: Qualitative: Descriptions of defects and anomalies encountered. Quantitative: Degree of fetal toxicity, and incidence of defects encountered.

Strengths: A fairly rapid test, 30 to 45 days to final report; Much historical background.

Weaknesses: Difficulty in assessing form and degree of fetal toxicity; Difficulty in extrapolation of data to human species.

Status of Development: Validated.

Describe: Standardized protocols have been available for over 10 years.

Applications: Multimedia.

Samples: Pure Chemicals: All classes. Complex Mixtures: Drinking water contaminants.

Duration: 45 days from initiation of treatment to completion of analysis.

Cost: $10,000/species.

Interpretation: A positive response in a species that has some metabolic similarities to man would suggest that the compound or mixture in question has the potential to induce abnormal development in humans.

Level of Complexity: 3.

OHEE Laboratory Involved: HERL-RTP, Environmental Biology Division, Neurobiology Branch.

Persons to Contact: N. Chernoff, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2326); R. Kavlock, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2326).

Grant/Contract Laboratory Involved and Principal Investigators: Contract currently under negotiation for the testing of 5 pesticides/year by established protocols.

Program Office Support: OPP; ORD.

References: 1) Pesticide Registration Guidelines. In preparation. 2) Testing of Chemicals for Carcinogenicity, Mutagenicity, and Teratogenicity. Published by Minister of Health and Welfare, Canada, 1973.

1304 DEVELOPMENTAL TOXICITY IN NEONATAL RATS

Biological Activity Detected: Developmental toxicity.

Principle: Pregnant rats are treated with compounds from the time of implantation continuing through lactation. The neonatal rats are then examined for a number of developmental milestones. This design allows for the continuous exposure of a mammalian organism through its most sensitive periods of development.

Endpoints: Qualitative: Growth and viability of neonatal rats. Quantitative: Measurement of developmental milestones in early postnatal life including reflex and morphological development; Also measurement of open field behavior in young adults.

Strengths: Exposure to organisms during the perinatal period maximizes the possibility of producing alterations in the morphological and behavioral aspects of the exposed animal.

Weaknesses: Testing is a labor-intensive operation; Lack of standardized procedures by various investigations; Difficult to extrapolate to humans.

Status of Development: Developmental.

Describe: Investigators in this field are evaluating the reliability and sensitivity of various test procedures.

Applications: Air; Water; Food.

Samples: Pure Chemicals: All classes. Complex Mixtures: N/A.

Duration: 3 to 4 months, start to report.

Cost: $10,000/species.

Interpretation: The production of growth disturbances/behavioral anomalies in the young postnatal animal is one of the more sensitive indicators of developmental toxicity. The implication of this test is that positive results may point to the induction of behavioral disturbances in humans subsequent to perinatal exposure. This link-up to human effects, however, has yet to be empirically demonstrated.

Level of Complexity: 3.

OHEE Laboratory Involved: HERL-RTP, Environmental Biology Division, Neurobiology Branch.

Persons to Contact: R. Kavlock, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2326); N. Chernoff, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2326).

Grant/Contract Laboratory Involved and Principal Investigators: N/A.

Program Office Support: OPP; ORD.

References: 1) Wiess, B., and J. Spyker. Behavioral Implications of Prenatal and Early Postnatal Exposure to Chemical Pollutants. Pediatrics, 53(5):Part III, May 1974. 2) Final Report of the Committee on Postnatal Evaluation of Animals Subjected to Insult During Development. C. Kimmel, Chairperson. Funded by the Nat. Inst. of Envir. Hlth. Sci., Mar. 1977.

1305 TERATOLOGY IN-VIVO/IN-VITRO

Biological Activity Detected: Teratology; Toxicology.

Principle: Treat pregnant rats from day 6 to 9 of gestation, then remove embryos and grow them in tissue culture for 24 to 48 hrs. During the culture interval, determinations of anatomical and biochemical growth are made.

Endpoints: Qualitative: Determine degree and normalacy of neuralation and somite development; Quantitative: Measure growth indicators, DNA, and protein.

Strengths: The maternal animal is treated; The embryo is accessible for a long enough period to make some measurements; Zero time measurements can use embryos from the same litter; Delays in growth during this gestational stage could result in malformations or abnormal development such as extra ribs; Embryos from the same litter are available for residue determination.

Weaknesses: The culture of the embryos is limited to 48 hours.

Status of Development: Being implemented.

Describe: Embryos from treated animals are being grown in culture. Methods for growing control embryos have been established.

Applications: Multimedia.

Samples: Pure Chemicals: All classes. Complex Mixtures: Ambient; Industrial; Energy Related; Transportation Related; Other.

Duration: 4 weeks, start to report.

Cost: $2,000/compound.

Interpretation: Toxic as well as teratogenic evaluators are determined for the embryo.

Level of Complexity: 3.

OHEE Laboratory Involved: HERL-RTP, Environmental Toxicology Division, Toxic Effects Branch, Perinatal Toxicology Section.

Persons to Contact: K.D. Courtney, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2370).

Grant/Contract Laboratory Involved and Principal Investigators: U. of Michigan, School of Medicine, Department of Anatomy, Ann Arbor, MI 48104, A. Beaudoin.

Program Office Support: OTS.

References: Not yet available.

1306 DIRECT SPECTRAL MEASUREMENT OF THE BIOCHEMICAL DEVELOPMENT OF THE NERVOUS SYSTEM

Biological Activity Detected: Delayed and arrested development.

Principle: A large change in the capacity of the nervous system for oxidative metabolism occurs with development. This increase may be followed directly in slices of tissue by taking advantage of the known spectral properties of the cytochromes using dual-wavelength spectroscopy. During brain development there are quantitative and qualitative differences in the way the tissue will respond to stimulation (e.g., electrical, elevated K^+) metabolically that may be observed using polarographic, spectral, and enzymatic analyses.

Endpoints: Qualitative: Developmental changes in: Cytochrome levels; Oxygen uptake in response to stimulation; Lactic acid output; Uptake and release of neurotransmitters; Amino acid concentrations; Redox changes produced in tissue pyridine nucleotides, flavoproteins, and cytochromes in response to standard stimulants. Quantitative: Measurements of: Cytochrome levels; Oxygen uptake in response to stimulation; Lactic acid output; Uptake and release of neurotransmitters; Amino acid concentrations; Redox changes produced in tissue pyridine nucleotides, flavoproteins, and cytochromes in response to standard stimulants.

Strengths: Spectral measurements can be applied to as little as 3 mg of tissue; Responses of tissues dependent upon the integrity of a wide variety of systems within the tissue (e.g., cell excitability, Na^+, K^+-ATPase, neurotransmittance, uptake, release and intrinsic activity, glycolytic and TCA cycle enzymes, etc.). The responses to K^+ are multiphasic, one phase probably being applicable to neuronal responses, the other to glial responses. Consequently, the system is unique in that it will detect a wide variety of types of damage.

Weaknesses: Principle weakness is that it does not lend itself to immediate identification of mechanism unless there is a direct effect on energy metabolism proper so that crossovers can be identified. However, this disadvantage is overcome by the fact that delays in development resulting from early exposure to Pb can be almost perfectly correlated with delays in morphological development (e.g., synaptogenesis).

Status of Development: Being implemented.

Describe: The basic developmental patterns for the cerebral cortex have been established for the spectral measurements of cytochrome concentrations and form of the metabolic responses. Spectral changes induced by K^+ have been correlated with respiratory changes, lactic acid output, and changes in tissue adenine nucleontides. Delays in development have been demonstrated in these parameters with Pb and correlated with delayed synaptogenesis in the rat.

Applications: Multimedia.

Samples: Pure Chemicals: Organics; Inorganics. Complex Mixtures: Industrial; Energy Related; Transportation Related; Other.

1306 DIRECT SPECTRAL MEASUREMENT OF THE BIOCHEMICAL DEVELOPMENT OF THE NERVOUS SYSTEM (continued)

Duration: As short as 3 weeks to as long as 3 months if animals are to be taken to sexual maturity.

Cost: $2,500 to $25,000, depending upon duration and number of replications.

Interpretation: Delays in biochemical development of the brain have been well correlated with delays in morphological development (synaptogenesis) and behavioral development (activity away from mother during lactation) in lead-treated animals. With these data as support, delayed biochemical development may be interpreted as presumptive evidence of retarded mental development.

Level of Complexity: 3.

OHEE Laboratory Involved: HERL-CIN, Laboratory Studies Division, Toxicological Assessment Branch.

Persons to Contact: R.J. Bull, U.S. EPA, HERL-CIN, 26 W. St. Clair St., Cincinnati, OH 45268, (FTS 684-7213).

Grant/Contract Laboratory Involved and Principal Investigators: In-house.

Program Office Support: OHEE.

References: 1) Himwich, W.A. Developmental Neurobiology. C.C. Thomas, Springfield, IL, 1970. pp. 22-46, 311-330, 331-369, 370-392. 2) Bull, R.J., and S.D. Lutkenhoff. J. Neurochem., 21:913-922, 1973. 3) Bull, R.J., and J.T. Cummins. J. Neurochem., 21:923-937, 1973. 4) Bull, R.J., and J.J. O'Neill. Psychopharmacol. Commun., 1:109-115, 1975. 5) Bull, R.J., P.M. Stanaszek, and S.D. Lutkenhoff. Envir. Hlth. Perspect., 12: 89-95, 1975. 6) Bull, R.J. J. Neurochem., 26:149-156, 1976.

CONTENTS: TEST SYSTEMS

2000 Short-Term Tests for Ecological Effects 107

2100 Freshwater and Marine Algae 107
2101 Freshwater Algal Assay Bottle Test. 108
2102 Marine Algal Assay Bottle Test. 109

2200 Freshwater Animals and Plants 111

2210 Fish Toxicity 111

2101 FRESHWATER ALGAL ASSAY BOTTLE TEST

Biological Activity Detected: Toxicity; Stimulation.

Principle: Standard test alga (Selenastrum capricornutum) is added to a test water, cultured under standard light, temperature, and gas exchange conditions, and evaluated for inhibitory (toxic) or stimulatory response to pollutant stress.

Endpoints: Qualitative: Can be used to screen stimulatory or inhibitory properties of point and non-point source pollutants. Quantitative: Response in mg dry wt l^{-1} of test alga either stimulatory (% S_{14}) or inhibited (% I_{14}) at day 14. Results are expressed as % stimulation or % inhibition as compared to control vs waste concentration.

Strengths: Detection of bioreactive components in a test water, or of waste discharge; identification of toxic or stimulatory components.

Weaknesses: Insufficient application of test to relate to potential health effects of a pollutant.

Status of Development: Validated.
Describe: N/A.

Applications: Water.

Samples: Pure Chemicals: Nutrients, NO_3, NO_2, NH_3, Ortho-P, Tot-P, Heavy metals, Pesticides, Herbicides, Insecticides. Complex Mixtures: Ambient - receiving water; Industrial - waste discharges; Energy Related - coal storage, leachates; Other - new product formulations, i.e., detergents.

Duration: Test: 14 days; Analysis: 5 days; Total: 21 days maximum in most cases.

Cost: $400 to evaluate a compound or complex waste.

Interpretation: Test data can be used to define nutrient limitation, heavy metal toxicity, and inhibitory or stimulatory properties of complex wastes, as they effect ecology of aquatic systems.

Level of Complexity: I.

OHEE Laboratory Involved: ERL-COR, Assessment Criteria Development Division, Special Studies Branch; ERL-DUL, Newtown Fish Toxicology Station.

Persons to Contact: W.E. Miller, U.S. EPA, ERL-COR, 200 SW 35th St., Corvallis, OR 97330, (FTS 420-4775, Commercial 503 757-4775); T. Shiroyama, U.S. EPA, ERL-COR, 200 SW 35th St., Corvallis, OR 97330, (FTS 420-4776, Commercial 503 757-4776); J.C. Greene, U.S. EPA, ERL-COR, 200 SW 35th St., Corvallis, OR 97330, (FTS 420-4764, Commercial 503 757-4764); E. Robinson, U.S. EPA, ERL-DUL, Newtown Fish Toxicology Station, 3411 Church St., Cincinnati, OH 45244, (FTS 684-8601).

Grant/Contract Laboratory Involved and Principal Investigators: N/A.

Program Office Support: OHEE; OTS.

References: 1) Algal Assay Procedure Bottle Test. U.S. EPA, Aug. 1971. 2) Standard Methods for the Examination of Water and Wastewater. 14th edition, 1975. To be published by ASTM.

2102 MARINE ALGAL ASSAY BOTTLE TEST

Biological Activity Detected: Toxicity; Stimulation.

Principle: Standard test algae are added to a test water, cultured under standard light, temperature, and gas exchange conditions and evaluated for inhibitory (toxic) or stimulatory response to pollutant stress.

Endpoints: Qualitative: Can be used to screen potential stimulatory or toxic properties of pollutants. Quantitative: Response in mg dry wt l^{-1} of the stimulatory (% S_{14}) or inhibitory (% I_{14}) response as compared to control vs control to which a waste concentration has been added.

Strengths: Detection of stimulatory and/or inhibitory bioreactive components in a test water, and of waste discharge.

Weaknesses: Insufficient application of test (round Robin); inadequate to predict possible health effects of specific pollutants.

Status of Development: Validated.

Describe: Interlaboratory calibration is necessary, as are broad application studies, i.e., complex wastes, organic compounds, etc.

Applications: Water.

Samples: Pure Chemicals: Nutrients, i.e., Nitrogen and Phosporous, Heavy metals, PCB's, Chloramines, Free chlorine. Complex Mixtures: Ambient - receiving waters; Industrial - waste discharges, dredge spoil, monochlorinated organics detergent builders.

Duration: Test: 14 days; Analysis: 5 days; Total: 21 days.

Cost: $400 to evaluate a compound of complex waste.

Interpretation: Test data can be used to define nutrient limitation, heavy metal toxicity, and inhibitory or stimulatory properties of complex wastes, as they effect ecology of marine ecosystems.

Level of Complexity: 1.

OHEE Laboratory Involved: ERL-NAR, Toxicology Branch, Marine Toxicology Team; ERL-COR, Marine and Freshwater Branch; ERL-GB, Experimental Environments Branch.

Persons to Contact: J. Gentile, U.S. EPA, ERL-NAR, South Ferry Rd., Narragansett, RI 02882, (FTS 838-4843 X244); D. Specht, ERL-COR, 200 SW 35th St., Corvallis, OR 97330, (FTS 420-4675); G. Walsh, U.S. EPA, ERL-GB, Sabine Island, Gulf Breeze, FL 32561 (FTS 686-9011).

Grant/Contract Laboratory Involved and Principal Investigators: N/A.

Program Office Support: OHEE; OTS.

References: 1) Marine Algal Assay Procedure Bottle Test. U.S. EPA, Dec. 1974. Bioassay Procedures for the Ocean Disposal Permit Program. EPA-600/9-78-010, U.S. EPA, 1978.

CONTENTS: TEST SYSTEMS

2200 Freshwater Animals and Plants 111

2210 Fish Toxicity. 111

2211 Acute Fish Toxicity Test, Static and Flow-Through, All Freshwater Species 112

2212 Subchronic Embryo-Larval, Early Juvenile Fish Toxicity Test 113

2213 Chronic Fish Toxicity Test, American Flagfish (*Jordanella floridae*) 114

2214 Fish Respiratory Activity Toxicity Test, Electrode Chamber Method 115

2215 Fish Avoidance Test, Gradient Tanks. 116

2220 Invertebrate Toxicity. 117

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2211 ACUTE FISH TOXICITY TEST, STATIC AND FLOW-THROUGH, ALL FRESHWATER SPECIES*

Biological Activity Detected: Toxicity.

Principle: Determine the 96-hr LC50 of various pure compounds and complex mixtures on freshwater organisms.

Endpoints: Qualitative: Behavior. Quantitative: Mortality (LC50).

Strengths: A "hard" number on lethality.

Weaknesses: The LC50 is not protective of aquatic species. Therefore, one must estimate or test for lower, no-effect concentrations.

Status of Development: Validated.

Describe: The 96-hr flow-through and static aquatic toxicity tests have been conducted for many years with some cross validation between laboratories; Some methods were written by Standard Methods and ASTM.

Applications: Water.

Samples: Pure Chemicals: Insecticides, Herbicides, Heavy Metals, PCB's. Complex Mixtures: Industrial - effluents; Energy Related - drilling muds, oils.

Duration: Test: 96 hours; Analysis: 96 hours.

Cost: Static test: $300; Flow-through test: $650.

Interpretation: From the tests the lethal effects of a toxicant along with a statistically valid 95% confidence interval can be determined.

Level of Complexity: I.

OHEE Laboratory Involved: ERL-DUL, Technical Assistance Branch.

Persons to Contact: C. Stephan, U.S. EPA, ERL-DUL, 6201 Congdon Blvd., Duluth, MN 55804 (FTS 783-9510, Commercial 218 727-6692 X570).

Grant/Contract Laboratory Involved and Principal Investigators: Bionomics, Inc., Wareham, MA, S. Sauter and K.J. Macek.

Program Office Support: OHEE; ORD.

References: 1) Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians. EPA-600/3-75-009, U.S. EPA, 1975.

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*This test is also applied to marine fish. See 2311.

2212 SUBCHRONIC EMBRYO-LARVAL, EARLY JUVENILE FISH TOXICITY TEST

Biological Activity Detected: Toxicity.

Principle: This test involves a 30-day exposure of the embryo-larval, early juvenile stages of development.

Endpoints: Qualitative: Behavior. Quantitative: Survival; Growth; Deformities; Determination of an estimated maximum acceptable toxicant concentration (MATC).

Strengths: Direct measure of an MATC; Gives an excellent estimate of chronic toxicity in 1 month. Chronic tests themselves would require 12 months to complete.

Weaknesses: Requires 30 days to complete test; Requires a supply of fish embryos.

Status of Development: Validated.

Describe: These tests have been run successfully by several contract laboratories as well as routinely by several EPA laboratories.

Applications: Water.

Samples: Pure Chemicals: Inorganics, Organics. Complex Mixtures: Industrial; Energy Related.

Duration: Test: 30 days; Analysis: Included in the 30 days.

Cost: $6,000/test.

Interpretation: This test gives an excellent estimate of the chronic (life-cycle) toxicity of individual toxicants or complex mixtures to fish.

Level of Complexity: 2.

OHEE Laboratory Involved: ERL-DUL, Research Branch, Physiological Effects of Toxicants Section.

Persons to Contact: J.M. McKim, U.S. EPA, ERL-DUL, 6201 Congdon Blvd., Duluth, MN 55804, (FTS 783-9567, Commercial 218 727-6692 X567); W.A. Brungs, U.S. EPA, ERL-DUL, 6201 Congdon Blvd., Duluth, MN 55804, (FTS 783-9546, Commercial 218 727-6692 X546).

Grant/Contract Laboratory Involved and Principal Investigators: Bionomics, Inc., Wareham, MA, S. Sauter and K.J. Macek.

Program Office Support: OHEE; ORD.

References: 1) McKim, J.M. Evaluation of Tests with the Early Life-Stage of Fish for Predicting Long-Term Toxicity. J. Fish Res. Bd. Can., 34(8):1148-1154, 1977. 2) U.S. EPA. Proposed Recommended Bioassay Procedure for Egg and Fry Stages of Freshwater Fish (manuscript). U.S. EPA, Duluth, MN. 3) Sauter, S., K.S. Buxton, K.J. Macek, and S.F. Petrocelli. Effects of Continuous Exposure to Lead, Chromium, Copper, and Cadmium on Eggs and Fry of Selected Freshwater Fish. Ecol. Res. Series, U.S. EPA, Duluth, MN, 1976.

2213 CHRONIC FISH TOXICITY TEST, AMERICAN FLAGFISH (JORDANELLA FLORIDAE)

Biological Activity Detected: Toxicity.

Principle: Determine the impact of toxicants on survival, growth, and reproduction of a freshwater fish with a rapid life cycle.

Endpoints: Qualitative: Behavior. Quantitative: Growth; Survival; Reproduction success (fecundity, hatchability); Determination of a maximum acceptable toxicant concentration (MATC).

Strengths: Similar in sensitivity to commonly tested freshwater fish, i.e., brook trout and fathead minnow; Short life cycle compared to most fish; Data generated on all stages of life cycle including those stages considered to be most sensitive.

Weaknesses: Fish is semitropical and may not have direct application to most U.S. waters.

Status of Development: Validated.

Describe: Many people at ERL-DUL have run this test successfully.

Applications: Water.

Samples: Pure Chemicals: Pesticides, Metals. Complex Mixtures: Waste oil.

Duration: Test: 6 months; Analysis: Included in 6 months.

Cost: $16,000.

Interpretation: This test can be used as an indicator of potential chronic fish effects.

Level of Complexity: 3.

OHEE Laboratory Involved: ERL-DUL, Research Branch, Physiological Effects of Toxicants Section.

Persons to Contact: W.A. Brungs, U.S. EPA, ERL-DUL, 6201 Congdon Blvd., Duluth, MN 55804, (FTS 783-9546, Commercial 218 727-6692 X546); R. Spehar, U.S. EPA, ERL-DUL, 6201 Congdon Blvd., Duluth, MN 55804, (FTS 783-9521, Commercial 218 727-6692 X521).

Grant/Contract Laboratory Involved and Principal Investigators: N/A.

Program Office Support: OHEE; ORD.

References: 1) Smith, W.E. A Cyprinodont Fish, *Jordanella floridae*, as a Reference Animal for Rapid Chronic Bioassays. J. Fish. Res. Bd. Can., 39:329-330, 1973. 2) Spehar, R.L. Cadmium and Zinc Toxicity to *Jordanella floridae*. J. Fish. Res. Bd. Can., 33:1939-1945, 1976. 3) U.S. EPA Committee on Aquatic Bioassays. Recommended Bioassay Procedures for Brook Trout, Bluegill, Fathead Minnow, and Flagfish Chronic Tests. U.S. EPA, Duluth, MN, 1972.

2214 FISH RESPIRATORY ACTIVITY TOXICITY TEST, ELECTRODE CHAMBER METHOD

Biological Activity Detected: Toxicity.
Principle: Bioelectric signals generated by respiratory activities are electronically amplified and recorded on stripchart records.
Endpoints: Gill purge (cough) and ventilation rates. Qualitative: Behavior. Quantitative: Rate of increase/unit of time.
Strengths: Rapid; Predictive.
Weaknesses: Stripchart records must be interpreted.
Status of Development: Being implemented.
Describe: The test results have been published (see references) and other researchers are starting to use this method.
Applications: Water.
Samples: Pure Chemicals: Multi. Complex Mixtures: Industrial.
Duration: Test: 4 days; Analysis: 1 day.
Cost: $750/chemical.
Interpretation: Concentrations which do not cause a statistically significant increase in gill purge rates are not likely to cause long-term adverse effects.
Level of Complexity: I.
OHEE Laboratory Involved: ERL-DUL, Research Branch, Physiological Effects of Pollutants Section.
Persons to Contact: R. Drummond, U.S. EPA, ERL-DUL, 6201 Congdon Blvd., Duluth, MN 55804, (FTS 783-9511, Commercial 218 727-6692 X511; R. Carlson, U.S. EPA, ERL-DUL, 6201 Congdon Blvd., Duluth, MN 55804, (FTS 783-9591, Commercial 218 727-6692 X591).
Grant/Contract Laboratory Involved and Principal Investigators: N/A.
Program Office Support: OHEE.
References: 1) Spoor, W.A., et al. Trans. Am. Fish. Soc., 1971. 2) Drummond, R., et al. J. Fish. Res. Bd. Can., 1973. 3) Drummond, R., et al. Trans. Am. Fish. Soc., 1974. 4) Carlson, R., and R. Drummond. Water Res., 1978. 5) Drummond, R., and R. Carlson. Ecol. Rep. Series, U.S. EPA, 1978.

2215 FISH AVOIDANCE TEST, GRADIENT TANKS

Biological Activity Detected: Toxicity; Physical environment.
Principle: Levels of environmental variables and toxicants avoided by fish are determined.
Endpoints: Qualitative: Avoidance behavior. Quantitative: Locomotor activity/unit of time.
Strengths: Short-term tests; Endpoints easy to determine; Collection of data can be automated.
Weaknesses: Needs validation.
Status of Development: Developmental.
Describe: Laboratory investigation underway.
Applications: Water.
Samples: Pure Chemicals: Dissolved gases, Oxygen, Metals, Organics. Complex Mixtures: Industrial; Energy Related.
Duration: Test: 4 hours; Analysis: 1 hour.
Cost: $150.
Interpretation: Avoidance has ecological significance.
Level of Complexity: 1.
OHEE Laboratory Involved: ERL-DUL, Office of the Director.
Persons to Contact: W.A. Spoor, U.S. EPA, ERL-DUL, 6201 Congdon Blvd., Duluth, MN 55804, (FTS 783-9506, Commercial 218 727-6692 X506).
Grant/Contract Laboratory Involved and Principal Investigators: N/A.
Program Office Support: ORD.
References: 1) Spoor, W.A., and R. Drummond. Trans. Am. Fish. Soc., 101:714-715, 1972.

CONTENTS: TEST SYSTEMS

2220 Invertebrate Toxicity. 117

  2221 Acute Invertebrate Toxicity Test, Static and Flow-Through, All Freshwater Species . . . 118

  2222 Subchronic Invertebrate Toxicity Test, Stream Insects 119

  2223 Subchronic Invertebrate Toxicity Tests, Chironomid (Tanytarsus dissimilis) 120

  2224 Chronic Invertebrate Toxicity Test, Water Flea (Daphnia magna) 121

2230 Plant Toxicity/Residue 123

2221 ACUTE INVERTEBRATE TOXICITY TEST, STATIC AND FLOW-THROUGH, ALL FRESHWATER SPECIES*

Biological Activity Detected: Toxicity.

Principle: Determine the 48-hr LC50 or EC50 of various pure compounds and complex mixtures on freshwater organisms.

Endpoints: Qualitative: Behavior. Quantitative: Mortality (LC50) or Immobilization EC50.

Strengths: A "hard" number on lethality, or measurable effect is determined.

Weaknesses: The LC50 or EC50 is not protective of aquatic species. Therefore, one must estimate or test for lower, no-effect concentrations.

Status of Development: Validated.

Describe: The 48-hr and 96-hr flow-through and static aquatic toxicity tests have been conducted for many years. Some methods were written by Standard Methods and ASTM.

Applications: Water.

Samples: Pure Chemicals: Insecticides, Herbicides, Heavy Metals, PCB's. Complex Mixtures: Industrial - effluents; Energy Related - drilling muds, oils.

Duration: Test: 48 or 96 hours; Analysis: 48 hours.

Cost: Static test: $300; Flow-through test: $650.

Interpretation: From the tests, the lethal effects of a toxicant along with a statistically valid 95% confidence interval can be determined.

Level of Complexity: I.

OHEE Laboratory Involved: ERL-DUL, Technical Assistance Branch; ERL-DUL, Extramural Program Branch.

Persons to Contact: C. Stephan, U.S. EPA, ERL-DUL, 6201 Congdon Blvd., Duluth, MN 55804 (FTS 783-9510, Commercial 218 727-6692 X570); K.E. Biesinger, U.S. EPA, ERL-DUL, 6201 Congdon Blvd., Duluth, MN 55804, (FTS 783-9524, Commercial 218 727-6692 X524).

Grant/Contract Laboratory Involved and Principal Investigators: Bionomics, Inc., Wareham, MA, S. Sauter and K.J. Macek.

Program Office Support: OHEE; ORD.

References: 1) Biesinger, K.E., and G. Christensen. Effects of Various Metals on Survival, Growth, Reproduction, and Metabolism of Daphnia magna. J. Fish Res. Bd. Can., 29(2):1691-1700, 1972. 2) Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians. EPA-600/3-75-009, U.S. EPA, 1975.

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*This test is also applied to marine invertebrates. See 2321.

2222 SUBCHRONIC INVERTEBRATE TOXICITY TEST, STREAM INSECTS

Biological Activity Detected: Toxicity.

Principle: The insects are exposed for 4 weeks. The time length allows for a good estimate of toxicity.

Endpoints: Qualitative: Behavior. Quantitative: Survival LC50; Bioaccumulation.

Strengths: Allows testing of stream invertebrates.

Weaknesses: Requires a "clean" collecting site near test facility; Requires good water source since it is a flow-through system.

Status of Development: Developmental.

Describe: System is developed and can be used if the need was established (i.e., could be implemented but isn't).

Applications: Water.

Samples: Pure Chemicals: Heavy metals, Pesticides. Complex Mixtures: Not tested.

Duration: Test: 4 week exposures; Analysis: Could be included in test time.

Cost: $2,800 at 4 weeks at $35,000/manyear.

Interpretation: It is a procedure which allows exposure for a time longer than acute (4-day) tests. This allows a better prediction of toxic effects.

Level of Complexity: 1 to 2.

OHEE Laboratory Involved: ERL-DUL, Research Branch, Physical Pollutant Section.

Persons to Contact: R.L. Anderson, U.S. EPA, ERL-DUL, 6201 Congdon Blvd., Duluth, MN 55804, (FTS 783-9565, Commercial 218 727-6692 X565).

Grant/Contract Laboratory Involved and Principal Investigators: N/A.

Program Office Support: OHEE; ORD.

References: 1) Spehar, R.L., R.L. Anderson, and J.T. Fiandt. Toxicity and Bioaccumulation of Cadmium and Lead in Aquatic Invertebrates. Envir. Pollut., 15:195, 1978. 2) Anderson, R.L., and D. DeFoe. Toxicity and Bioaccumulation of Endrin and Methoxychlor by Aquatic Invertebrates. 1979. In press.

2223 SUBCHRONIC INVERTEBRATE TOXICITY TEST, CHIRONOMID (TANYTARSUS DISSIMILIS)

Biological Activity Detected: Toxicity.

Principle: The animals are exposed from egg to 2nd or 3rd instar for 10 to 12 days of total exposure.

Endpoints: Qualitative: N/A. Quantitative: Survival; Growth; LC50 and EC values.

Strengths: Exposure through molting; Can also measure growth effects.

Weaknesses: Static, has only been tested with metals.

Status of Development: Developmental.

Describe: A report is being prepared regarding exposure to cadmium, lead, copper, and zinc.

Applications: Water.

Samples: Pure Chemicals: Heavy metals. Complex Mixtures: N/A.

Duration: Test: 10 to 12 days exposure; Analysis: Up to 2 weeks including preparation and clean-up.

Cost: 2 weeks at $35,000/manyear equals approximately $1,400; Chemical analysis might be additional if complex compounds were used.

Interpretation: The system exposes an insect during embryogenesis, hatching, growth, and molting. These 4 events are critical to the survival of the animal.

Level of Complexity: 2 to 3

OHEE Laboratory Involved: ERL-DUL, Research Branch, Physical Pollutant Section.

Persons to Contact: R.L. Anderson, U.S. EPA, ERL-DUL, 6201 Congdon Blvd., Duluth, MN 55804, (FTS 783-9565, Commercial 218 727-6692 X565).

Grant/Contract Laboratory Involved and Principal Investigators: N/A.

Program Office Support: OHEE; ORD.

References: None, in-house development.

2224 CHRONIC INVERTEBRATE TOXICITY TEST, WATER FLEA (DAPHNIA MAGNA)

Biological Activity Detected: Toxicity; Reproduction.

Principle: Animals are exposed for 3 to 4 weeks. The exposure period includes molting and reproduction.

Endpoints: Qualitative: N/A. Quantitative: Survival (LC50); Reproduction.

Strengths: Low equipment cost; Manpower requirements.

Weaknesses: Renewal system.

Status of Development: Being implemented.

Describe: Procedure has been published and is now being used with variation in many places.

Applications: Water.

Samples: Pure Chemicals: Metals, Organics. Complex Mixtures: Industrial; Energy Related.

Duration: Test: 3 to 4 weeks may be a good estimate. This would include data analysis and perhaps chemical analysis if single or low number mixtures are used.

Cost: 3 weeks at $35,000/manyear equals approximately $2,100.

Interpretation: Test allows fairly rapid screening of pollutants with a zooplankton representative.

Level of Complexity: 2.

OHEE Laboratory Involved: ERL-DUL, Technical Assistance Branch.

Persons to Contact: C. Stephan, U.S. EPA, ERL-DUL, 6201 Congdon Blvd., Duluth, MN 55804, (FTS 783-9510, Commercial 218 727-6692 X510).

Grant/Contract Laboratory Involved and Principal Investigators: N/A.

Program Office Support: OHEE; ORD.

References: 1) Biesinger, K., and G. Christensen. Effects of Various Metals on Survival, Growth, Reproduction and Metabolism of *Daphnia magna*. J. Fish. Res. Bd. Can., 29(12):1691-1700, 1972. 2) ASTM DRAFT. Proposed Standard Practice for Conducting Life-Cycle Toxicity Tests with the Daphnid, *Daphnia magna*.

CONTENTS: TEST SYSTEMS

2230 Plant Toxicity/Residue 123

  2231 Acute Plant Toxicity Test, Duckweed (_Lemma minor_) 124

2240 Ecosystem. 125

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2231 ACUTE PLANT TOXICITY TEST, DUCKWEED (LEMNA MINOR)

Biological Activity Detected: Toxicity; Residue.

Principle: Exposure of growing plants for 1 week.

Endpoints: Qualitative: N/A. Quantitative: Growth as reflected in frond count; EC values.

Strengths: Only flow-through system available for aquatic plants; Fast.

Weaknesses: Not developed to a point where weakness can be adequately described.

Status of Development: Developmental.

Describe: Exposure to copper has been completed. Exposure with other compounds is projected. Completion of procedure is projected for 1979.

Applications: Water.

Samples: Pure Chemicals: Metals, Organics. Complex Mixtures: Industrial; Energy Related.

Duration: Test: 1 week exposure, perhaps 1 to 2 weeks for preparation and clean-up.

Cost: 2 weeks at $35,000/manyear equals approximately $1,400; Analysis cost may be included if simple compounds are used.

Interpretation: This is the only system which allows exposure of an aquatic plant. Toxicity and bioaccumulation data should be obtainable from the procedure.

Level of Complexity: 1.

OHEE Laboratory Involved: ERL-DUL, Research Branch, Physical Pollutant Section.

Persons to Contact: R.L. Anderson, U.S. EPA, ERL-DUL, 6201 Congdon Blvd., Duluth, MN 55804, (FTS 783-9565, Commercial 218 727-6692 X565).

Grant/Contract Laboratory Involved and Principal Investigators: N/A.

Program Office Support: OHEE; ORD.

References: 1) Walbridge, C.T. A Flow-Through Testing Procedure with Duckweed (Lemna minor). Ecol. Res. Series, EPA 600/3-77-108, U.S. EPA, 1977.

CONTENTS: TEST SYSTEMS

2240 Ecosystem. 125
  2241 Laboratory Ecosystem 126

2300 Marine Animals 127

2310 Fish Toxicity. 127

2241 LABORATORY ECOSYSTEM

Biological Activity Detected: Toxicity.

Principle: To determine the effects of toxicants on ecosystem processes.

Endpoints: Qualitative: N/A. Quantitative: Modifications in rates and components of organic carbon budget includes water inflow and outflow; System photosynthesis and respiration; Periphyton growth; Benthic macroinvertebrates; Sediment accumulation and degradation; Macrophyte decomposition; MATC, based on change in the ecosystem processes.

Strengths: More realistic than single species testing; Includes species and environment interaction; Includes impact of environment on toxicant; Can follow accumulation of toxicants in food web; Includes some organisms not traditionally included in freshwater toxicology.

Weaknesses: Labor intensive; Difficult to obtain degree of replication necessary for statistical treatment of data; Complex interactions make data evaluation and interpretation difficult; Must extrapolate to natural situations.

Status of Development: Developmental.

Describe: Development of preliminary non-toxicant methods has just been initiated.

Applications: Water.

Samples: Pure Chemicals: Inorganics, Organics. Complex Mixtures: N/A.

Duration: Test: 3 to 5 months; Analysis: An additional 2 to 3 months.

Cost: $35,000.

Interpretation: Data can be used to identify sensitive ecosystem processes as well as to determine potential assimilation capacity.

Level of Complexity: 3.

OHEE Laboratory Involved: ERL-DUL, Newtown Fish Toxicology Station.

Persons to Contact: S.F. Hedtke, U.S. EPA, ERL-DUL, Newtown Fish Toxicology Station, 3411 Church Street, Cincinnati, OH 45244, (FTS 684-8601).

Grant/Contract Laboratory Involved and Principal Investigators: N/A.

Program Office Support: ORD.

References: Work is presently in developmental stages. Information on test system has not been published. See Persons to Contact.

CONTENTS: TEST SYSTEMS

2300 Marine Animals 127

  2310 Fish Toxicity. 127

    2311 Acute Fish Toxicity Test, Static and Flow-Through, All Marine Species 128

    2312 Subchronic Embryo-Larval Fish Toxicity Test, Sheepshead Minnow (*Cyprinodon variegatus*). . . . 129

    2313 Chronic Fish Toxicity Test, Sheepshead minnow (*Cyprinodon variegatus*) 130

  2320 Invertebrate Toxicity. 131

2311 ACUTE FISH TOXICITY TEST, STATIC AND FLOW-THROUGH, ALL MARINE SPECIES*

Biological Activity Detected: Toxicity.

Principle: Determine the 96-hr LC50 of various pure compounds and complex mixtures on marine organisms.

Endpoints: Qualitative: Behavior. Quantitative: Mortality (LC50).

Strengths: A "hard" number on lethality.

Weaknesses: The LC50 is not protective of aquatic species. Therefore, one must estimate or test for lower, no-effect concentrations.

Status of Development: Validated.

Describe: The 96-hr flow-through and static aquatic toxicity tests have been conducted for many years with cross validation by many laboratories; Some methods were written by Standard Methods and ASTM.

Applications: Water.

Samples: Pure Chemicals: Insecticides, Herbicides, Heavy Metals, PCB's. Complex Mixtures: Industrial - effluents; Energy Related - drilling muds, oils.

Duration: Test: 96 hours; Analysis: 96 hours.

Cost: Static test: $300; Flow-through test: $650.

Interpretation: From the tests the lethal effects of a toxicant along with a statistically valid 95% confidence interval can be determined.

Level of Complexity: I.

OHEE Laboratory Involved: ERL-DUL, Technical Assistance Branch.

Persons to Contact: C. Stephan, U.S. EPA, ERL-DUL, 6201 Congdon Blvd., Duluth, MN 55804 (FTS 783-9510, Commercial 218 727-6692 X570).

Grant/Contract Laboratory Involved and Principal Investigators: Bionomics, Inc., Wareham, MA, S. Sauter and K.J. Macek.

Program Office Support: OHEE; ORD.

References: 1) Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians. EPA-600/3-75-009, U.S. EPA, 1975.

*This test is also applied to freshwater fish. See 2211.

2312 SUBCHRONIC EMBRYO-LARVAL FISH TOXICITY TEST, SHEEPSHEAD MINNOW (*CYPRINODON VARIEGATUS*)

Biological Activity Detected: Toxicity; Growth; Pathologic effects.

Principle: To determine the effects of a toxicant on the early life stages of the sheepshead minnow.

Endpoints: Determine concentrations of a toxicant which affect survival, growth, behavior, and pathologic effects. *Qualitative*: Behavior. *Quantitative*: LC50 values; Significant differences can be established from experimental and control survival, and growth; Determination of an estimated maximum acceptable toxicant concentration (MATC).

Strengths: A good estimate of toxicity, particularly chronic toxicity, can be made in many instances.

Weaknesses: Duration is generally 28 days, or more.

Status of Development: Being implemented.

Describe: Embryo/fry studies have been conducted by ERL-GB and a private laboratory.

Applications: Water.

Samples: *Pure Chemicals*: Insecticides, Herbicides, PCB's, Pentachlorophenol. *Complex Mixtures*: N/A.

Duration: Test: 28 days; Analysis: 28 to 40 days if a chemical analysis is required for bioconcentration.

Cost: $6,000 to $7,000, depending upon whether or not chemical analyses are required.

Interpretation: From these tests the concentrations of a pollutant that affects survival, growth, etc. of a sensitive life stage of an estuarine fish can be determined.

Level of Complexity: 2.

OHEE Laboratory Involved: ERL-GB, Experimental Environments Branch.

Persons to Contact: *D.J. Hansen*, U.S. EPA, ERL-GB, Sabine Island, Gulf Breeze, FL 32561, (FTS 686-9011).

Grant/Contract Laboratory Involved and Principal Investigators: Bionomics, EG & G, Rt. 6, Box 1002, Pensacola, FL 32507, P.R. Parrish.

Program Office Support: OHEE; OPP; OWHM.

References: 1) Schimmel, S.C., P.R. Parrish, D.J. Hansen, J.M. Patrick, Jr., and J. Forester. Endrin: Effects on Several Estuarine Organisms. Proc. 28th Annu. Conf. Southeast. Asso. Game Fish Comm., 1974.

2313 CHRONIC FISH TOXICITY TEST, SHEEPSHEAD MINNOW (CYPRINODON VARIEGATUS)

Biological Activity Detected: Toxicity; Growth; Pathologic effects; Fecundity.

Principle: To determine the chronic effects of a pollutant on an estuarine fish.

Endpoints: Qualitative: Behavior. Quantitative: Growth; Survival; Fecundity; Pathologic effects.

Strengths: A good estimate of toxic affects.

Weaknesses: Cost; Time.

Status of Development: Being implemented.

Describe: Both ERL-GB and Bionomics, Pensacola, a private laboratory, have conducted the tests, although both laboratories have not completed full chronic tests on the same chemical for comparison.

Applications: Water.

Samples: Pure Chemicals: Insecticides, Herbicides. Complex Mixtures: N/A.

Duration: Test: 4 to 5 months; Analysis: 6 to 7 months.

Cost: $35,000 with analytical back-up.

Interpretation: One of the best available estimates of the effects of a pollutant on an estuarine fish is obtained.

Level of Complexity: 3.

OHEE Laboratory Involved: ERL-GB, Experimental Environments Branch.

Persons to Contact: D.J. Hansen, U.S. EPA, ERL-GB, Sabine Island, Gulf Breeze, FL 32561, (FTS 686-9011).

Grant/Contract Laboratory Involved and Principal Investigators: Bionomics, EG & G, Rt. 6, Box 1002, Pensacola, FL 32507. P.R. Parrish.

Program Office Support: OHEE; OPP; OWHM.

References: 1) Hansen, D.J., S.C. Schimmel, and J. Forester. Endrin: Effects on the Entire Life Cycle of a Salt Water Fish. J. Toxicol. Envir. Hlth., 3:721-733, 1977.

CONTENTS: TEST SYSTEMS

2320 Invertebrate Toxicity. 131
  2321 Acute Invertebrate Toxicity Test, Static and Flow-Through, All Marine Species 132
  2322 Acute Toxicity Test, Benthic Assemblages 133
  2323 Chronic Invertebrate Toxicity Test, Estuarine Shrimp (*Palaemonetes pugio*). 134
  2324 Chronic Invertebrate Toxicity Test, Estuarine mysid (*Mysidopsis bahia*) 135
2330 Ecosystem. 137

2321 ACUTE INVERTEBRATE TOXICITY TEST, STATIC AND FLOW-THROUGH, ALL MARINE SPECIES*

Biological Activity Detected: Toxicity.

Principle: Determine the 48-hr LC50 or EC50 of various pure compounds and complex mixtures on marine organisms.

Endpoints: Qualitative: Behavior. Quantitative: Mortality (LC50) or EC50.

Strengths: A "hard" number on lethality, or measurable effect, is determined.

Weaknesses: The LC50 or EC50 is not protective of aquatic species. Therefore, one must estimate or test for lower, no-effect concentrations.

Status of Development: Validated.

Describe: The 48-hr flow-through and static aquatic toxicity tests have been conducted for many years with cross validation by many laboratories. Some methods were written by Standard Methods and ASTM.

Applications: Water.

Samples: Pure Chemicals: Insecticides, Herbicides, Heavy Metals, PCB's. Complex Mixtures: Industrial - effluents; Energy Related - drilling muds, oils.

Duration: Test: 48 hours; Analysis: 48 hours.

Cost: Static test: $300; Flow-through test: $650.

Interpretation: From the tests the lethal effects of a toxicant along with a statistically valid 95% confidence interval can be determined.

Level of Complexity: I.

OHEE Laboratory Involved: ERL-DUL, Technical Assistance Branch.

Persons to Contact: C. Stephan, U.S. EPA, ERL-DUL, 6201 Congdon Blvd., Duluth, MN 55804 (FTS 783-9510, Commercial 218 727-6692 X510).

Grant/Contract Laboratory Involved and Principal Investigators: Bionomics, Inc., Wareham, MA, S. Sauter and K.J. Macek.

Program Office Support: OHEE; ORD.

References: 1) Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians. EPA-600/3-75-009, U.S. EPA, 1975.

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*This test is also applied to freshwater invertebrates. See 2221.

2322 ACUTE TOXICITY TEST, BENTHIC ASSEMBLAGES

Biological Activity Detected: Toxicity.

Principle: The acute effects of toxic substances on a macrofunal genthic microcosm representative of natural benthic assemblages in the Pacific Northwest will be examined.

Endpoints: Qualitative: Behavior, observe animals able to bury in dredge sediment. Quantitative: Count of survivors and count of animals able to bury in substrate in relation to water concentration provides EC values.

Strengths: Test organism is a sensitive amphipod species, Paraphoxus epistomus.

Weaknesses: New species not frequently used in aquatic testing.

Status of Development: Developmental.

Describe: This test procedure has only been conducted at the Newport, Oregon, facility.

Applications: Water; Soil.

Samples: Pure Chemicals: Heavy metals, i.e., Ca, Zn, Cr.

Complex Mixtures: Dredge sediment; Complex wastes.

Duration: Test: 96 hours.

Cost: Not yet determined.

Interpretaton: From this test one can determine if a dredge soil is acutely toxic to estuarine benthic animals.

Level of Complexity: I.

OHEE Laboratory Involved: ERL-COR, Ecological Effects Research Division, Marine and Freshwater Branch, Newport Field Station.

Persons to Contact: R. Swartz, U.S. EPA, ERL-COR, Newport Field Station, Marine Science Center, Newport, OR 97365, (FTS 423-4111, Commercial 503 867-4041).

Grant/Contract Laboratory Involved and Principal Investigators: N/A.

Program Office Support: OHEE; OTS.

References: 1) U.S. EPA, Corps of Engineers. Appendix F. Guidance for Performing Solid State Bioassays. In: Report on Ecological Evaluation of Proposed Discharge of Dredged Material on Ocean Waters. Tech. Committees on Criteria for Dredge and Fill Material. U.S. Army Waterways Station, Vicksburg, MS, July 1977.

2323 CHRONIC INVERTEBRATE TOXICITY TEST, ESTUARINE SHRIMP (PALAEMONETES PUGIO)

Biological Activity Detected: Toxicity; Growth; Reproduction.
Principle: Determine chronic effects of a pollutant on the entire life cycle of Grass Shrimp, *Palaemonetes pugio*.
Endpoints: Qualitative: N/A. Quantitative: LC50 values; Significant differences in survival, growth, and fecundity in experimental animals compared to controls.
Strengths: LC50 values; Significant differences in survival; Determination of growth and reproduction. Data give good indication of the effects of a toxicant over the animal's entire life cycle.
Weaknesses: Duration is long, about 5 months for chronic test; 2 to 3 months for partial-chronic.
Status of Development: Developmental.
Describe: Chronic tests have only been conducted at ERL-GB.
Applications: Water.
Samples: Pure Chemicals: Pesticides. Complex Mixtures: N/A.
Duration: Test: Approximately 5 months.
Cost: $25,000, including analytical back-up.
Interpretation: A maximum acceptable toxicant concentration can be established for this estuarine invertebrate.
Level of Complexity: 3.
OHEE Laboratory Involved: ERL-GB, Experimental Environments Branch.
Persons to Contact: D.B. Tyler-Schroeder, U.S. EPA, ERL-GB, Sabine Island, Gulf Breeze, FL 32561, (FTS 686-9011).
Grant/Contract Laboratory Involved and Principal Investigators: N/A.
Program Office Support: OHEE.
References: 1) Tyler-Schroeder, D.B. Use of the Grass Shrimp, *Palaemonetes pugio*, in a Life Cycle Toxicity Test. Symposium on Aquatic Toxicology and Hazard Evaluation. ASTM, 1978. In press. 2) Bioassay Procedures for the Ocean Disposal Permit Program. EPA-600/9-78-010, U.S. EPA, 1978.

2324 CHRONIC INVERTEBRATE TOXICITY TEST, ESTUARINE MYSID (MYSIDOPSIS BAHIA)

Biological Activity Detected: Toxicity; Fecundity; Growth.

Principal: Determine the chronic effects of toxicants on the entire life cycle of the crustacean species.

Endpoints: Qualitative: N/A. Quantitative: LC50 values; Significant differences in growth, reproduction, and survival.

Strengths: An estimate of chronic toxicity can be determined.

Weaknesses: Relatively high cost and duration of test; Animals are not always available throughout the year.

Status of Development: Being implemented.

Describe: ERL-GB and Bionomics EG & G, a private laboratory, have conducted these tests.

Applications: Water.

Samples: Pure Chemicals: Pesticides; Metals. Complex Mixtures: N/A.

Duration: Test: Approximately 28 days.

Cost: $7,000, including analytical back-up on pure chemicals.

Interpretation: A maximum acceptable toxicant concentration (MATC) can be established for a marine/estuarine invertebrate in this test.

Level of Complexity: 3.

OHEE Laboratory Involved: ERL-GB, Experimental Environments Branch.

Persons to Contact: D.W. Nimmo, U.S. EPA, ERL-GB, Sabine Island, Gulf Breeze, FL 32561, (FTS 686-9011).

Grant/Contract Laboratory Involved and Principal Investigators: Bionomics EG & G, Rt. 6, Box 1002, Pensacola, FL 32507, P.R. Parrish.

Program Office Support: OHEE; OWP.

References: 1) Nimmo, D.W., L.H. Bhaner, R.A. Rigby, J.M. Sheppard, and A.J. Wilson. *Mysidopsis bahia*: An Estuarine Species Suitable for Life-Cycle Toxicity Tests to Determine the Effects of a Pollutant. In: Aquatic Toxicology and Hazard Evaluation, Mayer, Hamelink, eds. ASTM, STP 634:109-116, 1977.

CONTENTS: TEST SYSTEMS

2330 Ecosystem. 137

2331 Estuarine Microcosym I 138

2332 Estuarine Microcosmy II. 139

2333 Estuarine Communities. 140

2400 Terrestrial Animals and Plants. 141

2410 Plants 141

Preceding page blank

2331 ESTUARINE MICROCOSMY I

Biological Activity Detected: Fate.

Principle: This test assesses transport and degradation potential of aquatic pollutants.

Endpoints: Qualitative: Types of transport; Localization; Mechanisms of degradation and character of degradation products. Quantitative: Rates of above processes; Effect of environmental parameters on rates; EC values.

Strengths: Use of environmental substrates; Mass balance analysis with radiolabeled pollutants; Versatility; Short turn-around time.

Weaknesses: Sealing factors from laboratory systems to the environment.

Status of Development: Developmental.

Describe: Systems have been designed and are in operation. Fate of pesticides has been tested. Optimization of systems are now in progress. Field validation is being initiated.

Applications: Water.

Samples: Pure Chemicals: Pesticides, Toxic Organics. Complex Mixtures: Industrial - effluents; Energy Related - oil.

Duration: Test: 4 to 8 weeks/chemical pollutant.

Cost: $2,000/month, not including senior investigator time.

Interpretation: This test supplies data on transport and degradation in natural system.

Level of Complexity: 2.

OHEE Laboratory Involved: ERL-GB, Processes and Effect Branch.

Persons to Contact: A.W. Bourquin, ERL-GB, Sabine Island, Gulf Breeze, FL 32561, (FTS 686-9011); R.L. Garnas, ERL-GB, Sabine Island, Gulf Breeze, FL 32561, (FTS 686-9011); P.H. Pritchard, ERL-GB, Sabine Island, Gulf Breeze, FL 32561, (FTS 686-9011).

Grant/Contract Laboratory Involved and Principal Investigators: N/A.

Program Office Support: OHEE; OPP; OTS; OEMI.

References: 1) Bourquin, A.W., R.L. Garnas, P.H. Pritchard, F.G. Wilkes, C.R. Cripe, and N.I. Rubinstein. Interdependent Microcosms for the Assessment of Pollutants in the Marine Environment. Internat. J. of Envir. Studies., 1978. In press.

2332 ESTUARINE MICROCOSMY II

Biological Activity Detected: Toxicity.
Principle: This test assesses the toxicity of pollutants to microbial growth and microbial degradation processes.
Endpoints: Qualitative: Types of microorganisms affected; Total biomass reduction; Selection of species; Physiological indies affected; Mechanisms of toxicity. Quantitative: EC values.
Strengths: Quick screen which uses natural assemblages of microorganisms.
Weaknesses: Extrapolation from laboratory systems to the environment; Requires analytical supports.
Status of Development: Being implemented.
Describe: Pesticides and toxic organics have been tested.
Applications: Water.
Samples: Pure Chemicals: Pesticides, Toxic organics, Heavy metals. Complex Mixtures: Industrial - effluents; Energy Related - oil.
Duration: Test: 4 to 8 weeks/chemical pollutant.
Cost: $1,500/month, not including senior investigator time.
Interpretation: This test determines toxicant effects on microbial assemblages.
Level of Complexity: 2.
OHEE Laboratory Involved: ERL-GB, Processes and Effect Branch.
Persons to Contact: A.W. Bourquin, ERL-GB, Sabine Island, Gulf Breeze, FL 32561, (FTS 686-9011); R.L. Garnas, ERL-GB, Sabine Island, Gulf Breeze, FL 32561, (FTS 686-9011); P.H. Pritchard, ERL-GB, Sabine Island, Gulf Breeze, FL 32561, (FTS 686-9011).
Grant/Contract Laboratory Involved and Principal Investigators: Georgia State U., Atlanta, GA 30303, D.G. Ahearn; Gulf Coast Research Laboratory, P.O. Box 26518, New Orleans, LA, W. W. Walker.
Program Office Support: OEMI.
References: 1) Bourquin, A.W., P.H. Pritchard, and W.R. Mahaffey. Effects of Kepone on Estuarine Microorganisms. Developments in Industrial Microbiology. 1978. In press. 2) Bourquin, A.W. Effects of Malathion on Microorganisms of an Artificial Salt-Marsh Environment. J. Envir. Quality, 6:383-378, 1977.

2333 ESTUARINE COMMUNITIES

Biological Activity Detected: Toxicity.

Principle: This test determines concentrations of a toxicant which offset the settling and development of benthic estuarine communities. These communities develop in sand substrate from larvae in unfiltered seawater.

Endpoints: Qualitative: N/A. Quantitative: Measure of species diversity; Number of sensitive phyla, biomass, growth, and total number of species; EC values.

Strengths: From this test we can see how a toxicant changes the community makeup by limiting sensitive groups and promoting growth of others.

Weaknesses: Duration is long (2 to 4 months); The investigator must have a good taxonomic background.

Status of Development: Developmental.

Describe: Only ERL-GB and one contractor have completed this type of community study.

Applications: Water.

Samples: Pure Chemicals: Pesticides. Complex Mixtures: Energy Related - drilling muds.

Duration: Test: 2 to 4 months to conduct test; Analysis: another 2 to 4 months to identify the animals.

Cost: $6,000.

Interpretation: From these studies we are able to predict the concentration of a toxicant that will adversely affect the recruitment and development of estuarine benthic communities.

Level of Complexity: 3 to 4.

OHEE Laboratory Involved: ERL-GB, Experimental Environments Branch.

Persons to Contact: M. Tagatz, ERL-GB, Sabine Island, Gulf Breeze, FL 32561, (FTS 686-9011).

Grant/Contract Laboratory Involved and Principal Investigators: Florida State U., Tallahassee, FL 32306, B. Glasson.

Program Office Support: OHEE; ORD.

References: 1) Hansen, D.J. Aroclor 1254: Effect on Composition of Developing Estuarine Animal Communities in the Laboratory. Marine Sci., 18:19-33, 1974.

CONTENTS: TEST SYSTEMS

2400 Terrestrial Animals and Plants. 141

  2410 Plants. 141

    2411 Stress Ethylene Bioassay in Plants. 142

    2412 Measurement of Nitrogenase Activity by Acetylene Reduction in Nodulated Plants 143

  2420 Ecosystem 145

2411 STRESS ETHYLENE BIOASSAY IN PLANTS

Biological Activity Detected: Alteration of physiological process.

Principle: Environmental stresses cause plants to produce large amounts of ethylene. The elevated ethylene occurs before and often in the absence of visual injury.

Endpoints: Qualitative: Observation of increase of ethylene production; In some cases modification of plant growth form.
Quantitative: Extent of increase for comparison with reference plants and chemicals.

Strengths: More sensitive than visual assessment; Less subject to variability.

Weaknesses: Short duration of the phenomena (approx. 48 hours); The test is designed as a flow-through test and not as a static system.

Status of Development: Being implemented.
Describe: The test has been used to examine effects of ozone and chlorine on a variety of plants ranging from pine trees to potatoes.

Applications: Air.

Samples: Pure Chemicals: Gaseous pollutants: Ozone, SO_2, NO_x, CO. Complex Mixtures: Ambient - air; Industrial - air pollutants; Energy Related - air pollutants; Transportation Related - air pollutants; Other - acid rain.

Duration: Test: 3 to 5 days exclusive of plant rearing; Analysis: Simultaneous with test.

Cost: $2,500.

Interpretation: This test provides evidence of tissue injury. Excess ethylene production may result in defoliation.

Level of Complexity: I.

OHEE Laboratory Involved: ERL-COR, Ecological Effects Research Division, Terrestrial Ecology Branch.

Persons to Contact: D.T. Tingey, ERL-COR, 200 SW 35th St., Corvallis, OR 97330, (FTS 420-4621).

Grant/Contract Laboratory Involved and Principal Investigators: N/A.

Program Office Support: OHEE.

References: 1) Tingey, D.T., C. Standley, and R.W. Field. Stress Ethylene Evolution, a Measure of Ozone Effects on Plants. Atmos. Envir., 10:969-974, 1976.

2412 MEASUREMENT OF NITROGENASE ACTIVITY BY ACETYLENE REDUCTION IN NODULATED PLANTS

Biological Activity Detected: Alteration of physiological process.
Principle: Measure reduction of acetylene to ethylene.
Endpoints: Qualitative: N/A. Quantitative: Amount of ethylene produced in relation to pollutant concentration.
Strengths: Requires minimal facilities or equipment; Rapid analysis.
Weaknesses: Needs further evaluation.
Status of Development: Being implemented.
Describe: This test is used to assess the impact of heavy metals (cadmium) on soybean, alder, and alfalfa nitrogen fixation systems.
Applications: Soil.
Samples: Pure Chemicals: Heavy metals. Complex Mixtures: Industrial - sludge.
Duration: Test: 10 to 15 days exclusive of time to grow plants; Analysis; Immediately following test.
Cost: $5,000/chemical.
Interpretation: This test provides information on the ability of nodulated plants to fix nitrogen in the presence of stress.
Level of Complexity: I.
OHEE Laboratory Involved: ERL-COR, Ecological Effects Research Division, Terrestrial Ecology Branch.
Persons to Contact: C. Wickliff, U.S. EPA, ERL-COR, 200 SW 35th St., Corvallis, OR 97330, (FTS 420-4622).
Grant/Contract Laboratory Involved and Principal Investigators: N/A.
Program Office Support: OHEE.
References: 1) Wickliff, C. PhD Thesis, Oregon State U. 1977. 2) Fishbeck, K., H.J. Evans, and L.L. Boersma. Agronomy J., 65:429-433, 1973. 3) Huang, Chi-Ying, F.A. Bazzaz, and L.N. Vanderhoff. Plant Physiology, 54:122-124, 1974.

CONTENTS: TEST SYSTEMS

2420 Ecosystem 145
    2421 Terrestrial Microcosm Chamber 146
    2422 Soil Core Microcosm 147
    2423 Soil/Litter Microcosm 148
2500 Physical-Chemical/Bio-accumulation. 149

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2421 TERRESTRIAL MICROCOSM CHAMBER

Biological Activity Detected: Toxicity; Bioaccumulation; Biomagnification; Community processes.

Principle: Radiolabeled pesticides at or below accepted field application rates are applied to assess fate, species and population effects.

Endpoints: Qualitative: Microcosms should be viewed as a tool which at present provide only trends in fate or effects. Quantitative: N/A.

Strengths: Higher link between bench and field; Lower cost than field studies; Provides indices of distribution.

Weaknesses: Cost/unit; Ambiguity of results; Not validated.

Status of Development: Developmental.

Describe: At present ERL-COR is developing a testing protocol consisting of not one system, but a methodology that utilizes "benchmark" data as well as various microcosms depending on the information required.

Applications: Air; Water; Soil.

Samples: Pure Chemicals: Heavy metals, Pesticides, Gaseous pollutants. Complex Mixtures: Industrial - effluents; Energy Related - air pollutants; Transportation Related - air pollutants.

Duration: Test: 2 months; Analysis: 3 months.

Cost: $50 to $100,000, depending on the compound.

Interpretation: Fate results utilized ecological mag: bioaccumulation, biodegradability and degradation. Effects' results are still open to discussion other than acute toxicity.

Level of Complexity: 3.

OHEE Laboratory Involved: ERL-COR, Ecological Effects Research Division, Terrestrial Ecology Branch.

Persons to Contact: J.D. Gile, U.S. EPA, ERL-COR, 200 SW 35th St., Corvallis, OR 97330, (FTS 420-4649); J.W. Gillett, U.S. EPA, ERL-COR, 200 SW 35th St., Corvallis, OR 97330, (FTS 420-4622).

Grant/Contract Laboratory Involved and Principal Investigators: U. of Michigan, Ann Arbor, MI 48104, E. Goodman; U. of Wisconsin, Madison, Wisconsin 53706, P. Lichtenstein.

Program Office Support: OHEE; OPP.

References: 1) Gillett, J.W., and J.D. Gile. Pesticide Fate in Terrestrial Laboratory Ecosystems. Intern. J. Envir. Studies, 10:15-22, 1976.

2422 SOIL CORE MICROCOSM

Biological Activity Detected: Monitor community processes.

Principle: The use of an intact system provides a more realistic representation of a natural system. The test relies on the production of CO_2 and nutrient loss as indicators of community fitness.

Endpoints: Qualitative: N/A. Quantitative: Rate of CO_2 production; Loss of nutrients through leachate.

Strengths: Small size; Low cost/unit; Simple design.

Weaknesses: High degree of variability between units, due to natural variability.

Status of Development: Developmental.

Describe: To date, only inorganics have been examined in the soil core. ERL-COR is currently evaluating the system for use with a broad spectrum of toxics.

Applications: Soil.

Samples: Pure Chemicals: Heavy metals, Pesticides. Complex Mixtures: Industrial - waste effluents.

Duration: Test: 3 months; Analysis: 1 to 2 months.

Cost: $2,000 to $3,000.

Interpretation: This test reflects the ability of a community to decompose material. It also reflects the impact of a chemical on nutrient cycling.

Level of Complexity: 3.

OHEE Laboratory Involved: ERL-COR, Ecological Effects Research Division, Terrestrial Ecology Branch.

Persons to Contact: J. Gile, U.S. EPA, ERL-COR, 200 SW 35th St., Corvallis, OR 97330, (FTS 420-4649).

Grant/Contract Laboratory Involved and Principal Investigators: N/A.

Program Office Support: OTS.

References: 1) Draggon, S. Soil Core Microcosm. Proceedings of EPA Workshop on Terrestrial Microcosms. J.W. Gillett, ed. Corvallis, OR, 1977. In press.

2423 SOIL/LITTER MICROCOSM

Biological Activity Detected: Monitor decomposition process.

Principle: Carbon dioxide production and O_2 consumption are monitored as indicators of microbial respiration.

Endpoints: Qualitative: O_2; CO_2; Microbiota; Microarthropods; Nematodes. Quantitative: Change in respiratory rate with changing chemical concentration.

Strengths: Low cost/unit; Simple analytical technique and equipment; Overall simplicity of system.

Weaknesses: Limited type of data generated.

Status of Development: Developmental.

Describe: Soil is tested in 1 qt. mason jars with respiration CO_2 and O_2 measured by simple titrametric or gasometric techniques.

Applications: Soil.

Samples: Pure Chemicals: Heavy metals, Pesticides. Complex Mixtures: Ambient; Industrial; Energy Related.

Duration: Test: 1 to 4 weeks; Analysis: Simultaneous with test.

Cost: $500/chemical.

Interpretation: Changes in respiration may reflect the ability of a community to decompose organic matter.

Level of Complexity: 3.

OHEE Laboratory Involved: ERL-COR, Ecological Effects Research Division, Terrestrial Ecology Branch.

Persons to Contact: B. Lighthart, U.S. EPA, ERL-COR, 200 SW 35th St., Corvallis, OR 97330, (FTS 420-4832).

Grant/Contract Laboratory Involved and Principal Investigators: N/A.

Program Office Support: OHEE.

References: 1) Soil/Litter Microcosm. In: Proceedings of EPA Workshop on Terrestrial Microcosms. J.W. Gillett, ed. U.S. EPA, Corvallis, OR, 1977. In press.

CONTENTS: TEST SYSTEMS

2500 Physical-Chemical/Bio-accumulation. 149

  2501 Bioconcentration Studies, Marine and Freshwater Animals. 150

  2502 Reverse-Phase High Pressure Liquid Chromatography (HPLC) 151

Indices. 153

2501 BIOCONCENTRATION STUDIES, MARINE AND FRESHWATER ANIMALS

Biological Activity Detected: Bioconcentration.

Principle: To determine the uptake and depuration rates of pure chemicals in tissues.

Endpoints: Qualitative: Flesh tainting. Quantitative: Uptake rates; Depuration rates; Bioconcentration factors (uptake of a pure chemical in tissues divided by the concentration of the chemical in the exposure water).

Strengths: A good estimate of the uptake, depuration rates of a pure chemical, plateau level, and bioconcentration factors.

Weaknesses: Duration at least 1 month; Requires complicated chemical analyses; Does not always reflect a true picture of bioconcentration in the field because of uptake from other sources (of food and sediment).

Status of Development: Being implemented.

Describe: Various laboratories within EPA and private industry are now using this method. The procedure has not yet been accepted by ASTM as it is still being revised.

Applications: Water.

Samples: Pure Chemicals: Insecticides, Herbicides, PCB's, Chlorinated hydrocarbons, Radiolabeled material. Complex Mixtures: N/A.

Duration: Test: 30 to 60 days; Analysis: 30 to 60 days.

Cost: $4,000.

Interpretation: From the above tests the potential of contaminants reaching man's seafoods can be determined.

Level of Complexity: 2.

OHEE Laboratory Involved: ERL-DUL, Research Branch, Chemical Pollutant Section; ERL-GB, Experimental Environments Branch.

Persons to Contact: G.D. Veith, ERL-DUL, 6201 Congdon Blvd., Duluth, MN 55804, (FTS 783-9534); S.C. Schimmel, ERL-GB, Sabine Island, Gulf Breeze, FL 32561, (FTS 686-9011).

Grant/Contract Laboratory Involved and Principal Investigators: Bionomics, EG & G, Rt. 6, Box 1002, Pensacola, FL 32507, P.R. Parrish.

Program Office Support: OHEE; OPP.

References: 1) ASTM Committee on Bioconcentration of Test Materials in Fishes and Oysters. (E-35 Committee on Pesticides). In preparation.

2502 REVERSE-PHASE HIGH PRESSURE LIQUID CHROMATOGRAPHY (HPLC)

Biological Activity Detected: Bioconcentration.

Principle: HPLC retention time correlates with the logarithm of the partition coefficient which correlates with the bioconcentration factor of organic chemicals in fish tissue.

Endpoints: Qualitative: N/A. Quantitative: Provides bioconcentration potential of organic chemicals in animal tissue.

Strengths: Rapid; Inexpensive.

Weaknesses: Requires a HPLC.

Status of Development: Being implemented.

Describe: Chemical analysis is utilizing HPLC to obtain Log P values, which can be correlated with water solubility and bioconcentration factors for organic chemicals.

Applications: Water.

Samples: Pure Chemicals: Organic; Inorganic. Complex Mixtures: Industrial.

Duration: Test: 10 to 20 min; Analysis: 10 to 20 min.

Cost: $100.

Interpretation: This work sets forth a rapid, inexpensive method for screening chemicals for their bioconcentration potential in the environment.

Level of Complexity: 0.

OHEE Laboratory Involved: ERL-DUL, Research Branch, Physiological Effects of Pollutants Section.

Persons to Contact: G.D. Veith, U.S. EPA, ERL-DUL, 6201 Congdon Blvd., Duluth, MN 55804, (FTS 783-9534, Commercial 218 727-6692 X534).

Grant/Contract Laboratory Involved and Principal Investigators: N/A.

Program Office Support: OHEE; ORD.

References: 1) Veith, G.D., and Morris. Ecol. Rept. Series, U.S. EPA, 1978. In press. 2) Lee, Huges, and G.D. Veith. Water, Air, and Soil Pollution, 8:749-484, 1977. 3) Veith, G.D., and N.M. Austin. Detection and Isolation of Bioaccumulable Chemicals in Complex Effluents. In: Identification and Analysis of Organic Pollutants in Water. L.A. Keith, ed. Ann Arbor Science Publishers, Ann Arbor, MI, 1976. pp. 297-304.

INDICES

Preceding page blank

INDEX I: TEST SYSTEMS IN NUMERICAL ORDER

TEST SYSTEM NUMBER	NAME OF TEST SYSTEM	PAGE
1111	Integrated System: Lung Organ Culture System, Tissue Homogenates, Purified Enzyme Systems.	2
1112	Integrated System: Tissue Homogenates, Purified Enzyme Systems	3
1113	Instrumental Methods of Detecting Functional and Metabolic Damage to Target Tissues	4
1114	Tracheal Mucociliary Transport	6
1115	Platelet Secretion Measured by ATP Release	8
1116	Mammalian Platelet and Fibroblast Impairment	9
1117	Lymphocyte Cytotoxicity.	10
1118	Impairment of Neutrophil Phagocytosis.	11
1119	Human Lung Fibroblasts (WI38).	12
11110	Chinese Hamster Ovary (CHO) Clonal Toxicity.	14
11111	Rabbit Alveolar Macrophage (RAM)	16
11112	Rat Hepatocyte (Liver Cell).	18
11113	Chinese Hamster Ovary (CHO) Cytotoxicity and Mutagenicity	19
1121	LD50	22
1122	Whole Animal LD50 — Oral, Dermal	23
1131	Integrated System: General Clinical Pathology	26
1132	Sleep-Time Study	27
1133	Metabolism of Chlorinated Hydrocarbons in Subhuman Primates	28

(continued)

INDEX I: TEST SYSTEMS IN NUMERICAL ORDER (continued)

TEST SYSTEM NUMBER	NAME OF TEST SYSTEM	PAGE
1134	Metabolic Profiles	29
1135	Model Substrate Metabolism	30
1136	Xenobiotic Mechanisms	32
1137	Oxidant Production by Leukocytes and Alveolar Macrophages Measured by Chemiluminescence	33
1138	Cyclic Nucleotide Concentrations in Leukocytes and Alveolar Macrophages	34
1141	Toxicity of Aerosolized Pollutants/Acute and Subacute	36
1142	Toxicity of Gaseous and Vapor Phase/Acute and Subacute	37
1143	Inhalation LC50 Tests	38
1144	Deposition and Clearance of Radioactive Materials Following Inhalation Exposure	39
1145	Integrated System: Pulmonary Function and Pulmonary Metabolism	40
1146	Pulmonary Function in Rats	41
1147	Pulmonary Function of Unanesthetized Guinea Pigs	42
1148	Arterial Blood Gas Measurement in Conscious Rats	43
1149	Infectivity Model	44
11410	In-Vivo Alveolar Macrophage Cytotoxicity	45
1151	Integrated System: Neurobehavioral Toxicological Assessment	48
1152	Integrated System: The Effects of Selected Organic Contaminants in Drinking Water on the Functions of the Reproductive, Nervous, and Immune Systems	49
1153	Computer Automated Analysis of Patterned Behavior in the Primate	51

(continued)

INDEX I: TEST SYSTEMS IN NUMERICAL ORDER (continued)

TEST SYSTEM NUMBER	NAME OF TEST SYSTEM	PAGE
1154	Computer Automated Analysis of Learning and Memory in the Primate	52
1155	Integrated System: Behavioral Analysis of Rats — Developmental, Locomotor, Exploratory, and Learned Behavior	53
1156	Integrated System: Instrumental Methods of Detecting Functional and Metabolic Damage to the Nervous System	54
1211	*Salmonella typhimurium* (Ames)	58
1212	*Escherichia coli* (WP2)	60
1213	*Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, Forward and Reverse Mutation	61
1214	Body Fluid Analysis	62
1215	Bacterial Plasmids	63
1216	Mouse Lymphoma (L5178Y)	64
1217	Chinese Hamster Ovary Cells (CHO) Drug Resistance	65
1218	Chinese Hamster Cells (CHO) Nutritional Competency	66
1219	Chinese Hamster Lung Cells (V79)	67
12110	*Drosophila melanogaster*, Sex Linked Recessive Lethal	68
12111	*Tradescantia* Stamen Hair	69
12112	Maize Waxy Locus Assay	70
12113	In-Vivo Cytogenetics in Mice	71
1221	*Bacillus subtilis* rec$^-$	74
1222	*Escherichia coli*, Pol A$^-$	75
1223	Mitotic Recombination and Gene Conversion in *Saccaromyces cerevisiae*	76

(continued)

INDEX I: TEST SYSTEMS IN NUMERICAL ORDER (continued)

TEST SYSTEM NUMBER	NAME OF TEST SYSTEM	PAGE
1224	Unscheduled DNA Synthesis (UDS)	77
1225	Sister-Chromatid Exchange Formation (SCE)	78
1226	In-Vivo Assessment of DNA Damage	79
1227	Intact Rodent Hepatocytes in Primary Culture	80
1228	In-Vivo DNA Binding	81
1229	Chinese Hamster Cells (CHO) Unscheduled DNA Synthesis (UDS)	82
1231	C3H10T1/2CL8 Mouse Embryo Fibroblast Oncogenic Transformation with Exogenous Metabolic Activation	84
1232	C3H10T1/2CL8 Mouse Embryo Fibroblast Oncogenic Transformation	85
1233	BHK-21 Mammalian Cell Oncogenic Transformation	86
1234	Syrian Hamster Embryo Oncogenic Transformation (Focus Assay)	87
1235	BALB 3T3 Oncogenic Transformation and Mutagenesis with Exogenous Metabolic Activation	88
1236	BALB 3T3 Oncogenic Transformation	89
1237	Pulmonary Adenoma Bioassay in Mice	90
1241	Tumor Induction in Massive Crustaceans, Molluscs, and Teliost Fish	92
1242	MFO Induction as an Indicator of Toxicity Exposure	93
1243	Limb Regeneration System	94
1244	Isogenic Fish	95
1245	Integrated System: Development of Mutagen/Carcinogen Activation, Concentration, Separation, and Weathering Systems	96
1246	Biphenyl Hydroxylase	97

(continued)

INDEX I: TEST SYSTEMS IN NUMERICAL ORDER (continued)

TEST SYSTEM NUMBER	NAME OF TEST SYSTEM	PAGE
1301	Mammalian Teratology	100
1302	Perinatal Toxicology	101
1303	Fetal Toxicity in Rats, Mice, Guinea Pigs/ Hamsters	102
1304	Developmental Toxicity in Neonatal Rats	103
1305	Teratology In-Vivo/In-Vitro	104
1306	Direct Spectral Measurement of the Biochemical Development of the Nervous System	105
2101	Freshwater Algal Assay Bottle Test	108
2102	Marine Algal Assay Bottle Test	109
2211	Acute Fish Toxicity Test, Static and Flow-Through, All Freshwater Species	112
2212	Subchronic Embryo-Larval, Early Juvenile Fish Toxicity Test	113
2213	Chronic Fish Toxicity Test, American Flagfish (*Jordanella floridae*)	114
2214	Fish Respiratory Activity Toxicity Test, Electrode Chamber Method	115
2215	Fish Avoidance Test, Gradient Tanks	116
2221	Acute Invertebrate Toxicity Test, Static and Flow-Through, All Freshwater Species	118
2222	Subchronic Invertebrate Toxicity Test, Stream Insects	119
2223	Subchronic Invertebrate Toxicity Test, Chironomid (*Tanytarsus dissimilis*)	120
2224	Chronic Invertebrate Toxicity Test, Water Flea (*Daphnia magna*)	121
2231	Acute Plant Toxicity Test, Duckweed (*Lemma minor*)	124
2241	Laboratory Ecosystem	126

(continued)

INDEX I: TEST SYSTEMS IN NUMERICAL ORDER (continued)

TEST SYSTEM NUMBER	NAME OF TEST SYSTEM	PAGE
2311	Acute Fish Toxicity Test, Static and Flow-Through, All Marine Species	128
2312	Subchronic Embryo-Larval Fish Toxicity Test, Sheepshead Minnow (*Cyprinodon variegatus*)	129
2313	Chronic Fish Toxicity Test, Sheepshead Minnow (*Cyprinodon variegatus*)	130
2321	Acute Invertebrate Toxicity Test, Static and Flow-Through, All Marine Species	132
2322	Acute Toxicity Test, Benthic Assemblages	133
2323	Chronic Invertebrate Toxicity Test, Estuarine Shrimp (*Palaemonetes pugio*)	134
2324	Chronic Invertebrate Toxicity Test, Estuarine Mysid (*Mysidopsis bahia*)	135
2331	Estuarine Microcosmy I	138
2332	Estuarine Microcosmy II	139
2333	Estuarine Communities	140
2411	Stress Ethylene Bioassay in Plants	142
2412	Measurement of Nitrogenase Activity by Acetylene Reduction in Nodulated Plants	143
2421	Terrestrial Microcosm Chamber	146
2422	Soil Core Microcosm	147
2423	Soil/Litter Microcosm	148
2501	Bioconcentration Studies, Marine and Freshwater Animals	150
2502	Reverse-Phase High Pressure Liquid Chromatography (HPLC)	151

INDEX II: TEST SYSTEMS IN ALPHABETICAL ORDER*

NAME OF TEST SYSTEM	TEST SYSTEM NUMBER	PAGE
Acute Fish Toxicity Test, Static and Flow-Through, All Freshwater Species	2211	112
Acute Fish Toxicity Test, Static and Flow-Through, All Marine Species	2311	128
Acute Invertebrate Toxicity Test, Static and Flow-Through, All Freshwater Species	2221	118
Acute Invertebrate Toxicity Test, Static and Flow-Through, All Marine Species	2321	132
Acute Plant Toxicity Test, Duckweed (*Lemma minor*)	2231	124
Acute Toxicity Test, Benthic Assemblages	2322	133
Algal Assay Bottle Test, Freshwater†	2101	108
Algal Assay Bottle Test, Marine†	2102	109
Alveolar Macrophage Cytotoxicity, In-Vivo†	11410	45
(Ames) or *Salmonella typhimurium*†	1211	58
Arterial Blood Gas Measurement in Conscious Rats	1148	43
Bacillus subtilis rec^{-}	1221	74
Bacterial Plasmids	1215	63
BALB 3T3 Oncogenic Transformation	1236	89
BALB 3T3 Oncogenic Transformation and Mutagenesis with Exogenous Metabolic Activation	1235	88

(continued)

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*Integrated Systems are listed twice: once under "Integrated Systems" and once under the title proper.

†This test is listed under two titles.

INDEX II: TEST SYSTEMS IN ALPHABETICAL ORDER (continued)

NAME OF TEST SYSTEM	TEST SYSTEM NUMBER	PAGE
Behavioral Analysis of Rats — Developmental, Locomotor, Exploratory, and Learned Behavior: Integrated System	1155	53
BHK-21 Mammalian Cell Oncogenic Transformation	1233	86
Bioconcentration Studies, Marine and Freshwater Animals	2501	150
Biphenyl Hydroxylase	1246	97
Body Fluid Analysis	1214	62
C3H10T1/2CL8 Mouse Embryo Fibroblast Oncogenic Transformation	1232	85
C3H10T1/2CL8 Mouse Embryo Fibroblast Oncogenic Transformation with Exogenous Metabolic Activation	1231	84
Chinese Hamster Cells (CHO) Nutritional Competency	1218	66
Chinese Hamster Cells (CHO) Unscheduled DNA Synthesis (UDS)	1229	82
Chinese Hamster Lung Cells (V79)	1219	67
Chinese Hamster Ovary Cells (CHO) Clonal Toxicity Assay	11110	14
Chinese Hamster Ovary Cells (CHO) Cytotoxicity and Mutagenicity Assay	11113	19
Chinese Hamster Ovary Cells (CHO) Drug Resistance	1217	65
Chronic Fish Toxicity Test, American Flagfish (Jordanella floridae)	2213	114
Chronic Fish Toxicity Test, Sheepshead Minnow (Cyprinodon variegatus)	2313	130
Chronic Invertebrate Toxicity Test, Estuarine Mysid (Mysidopsis bahia)	2324	135

(continued)

INDEX II: TEST SYSTEMS IN ALPHABETICAL ORDER (continued)

NAME OF TEST SYSTEM	TEST SYSTEM NUMBER	PAGE
Chronic Invertebrate Toxicity Test, Estuarine Shrimp (*Palaemonetes pugio*)	2323	134
Chronic Invertebrate Toxicity Test, Water Flea (*Daphnia magna*)	2224	121
Computer Automated Analysis of Learning and Memory in the Primate	1154	52
Computer Automated Analysis of Patterned Behavior in the Primate	1153	51
Cylic Nucleotide Concentrations in Leukocytes and Alveolar Macrophages	1138	34
Cytogenetics in Mice, In-Vivo†	12113	71
Deposition and Clearance of Radioactive Materials Following Inhalation Exposure	1144	39
Development of Mutagen/Carcinogen Activation, Concentration, Separation, and Weathering Systems: Integrated System	1245	96
Developmental Toxicity in Neonatal Rats	1304	103
Direct Spectral Measurement of the Biochemical Development of the Nervous System	1306	105
DNA Binding, In-Vivo†	1228	81
DNA Damage, In-Vivo Assessment††	1226	79
Drosophila melanogaster, Sex Linked Recessive Lethal	12110	68
Effects of Selected Organic Contaminants in Drinking Water on the Functions of the Reproductive, Nervous, and Immune Systems: Integrated System	1152	49
Escherichia coli (WP2)	1212	60
Escherichia coli, Pol A^-	1222	75
Estuarine Communities	2333	140
Estuarine Microcosmy I	2331	138

(continued)

INDEX II: TEST SYSTEMS IN ALPHABETICAL ORDER (continued)

NAME OF TEST SYSTEM	TEST SYSTEM NUMBER	PAGE
Estuarine Microcosmy II	2332	139
Fetal Toxicity in Rats, Mice, Guinea Pigs/Hamsters	1303	102
Fish Avoidance Test, Gradient Tanks	2215	116
Fish Respiratory Activity Toxicity Test, Electrode Chamber Method	2214	115
Freshwater Algal Assay Bottle Test†	2101	108
General Clinical Pathology: Integrated System	1131	26
Human Lung Fibroblasts (WI38)	1119	12
Impairment of Neutrophil Phagocytosis	1118	11
Infectivity Model	1149	44
Inhalation LC50 Tests	1143	38
Instrumental Methods of Detecting Functional and Metabolic Damage to the Nervous System: Integrated System	1156	54
Instrumental Methods of Detecting Functional and Metabolic Damage to Target Tissues	1113	4
Intact Rodent Hepatocytes on Primary Culture	1227	80
Integrated System: Behavioral Analysis of Rats — Developmental, Locomotor, Exploratory, and Learned Behavior	1155	53
Integrated System: Development of Mutagen/Carcinogen Activation, Concentration, Separation and Weathering Systems	1245	96
Integrated System: The Effects of Selected Organic Contaminants in Drinking Water on the Functions of the Reproductive, Nervous, and Immune Systems	1152	49
Integrated System: General Clinical Pathology	1131	26

(continued)

INDEX II: TEST SYSTEMS IN ALPHABETICAL ORDER (continued)

NAME OF TEST SYSTEM	TEST SYSTEM NUMBER	PAGE
Integrated System: Instrumental Methods of Detecting Functional and Metabolic Damage to the Nervous System	1156	54
Integrated System: Lung Organ Culture System, Tissue Homogenates, Purified Enzyme Systems	1111	2
Integrated System: Neurobehavioral Toxicological Assessment	1151	48
Integrated System: Pulmonary Function and Pulmonary Metabolism	1145	40
Integrated System: Tissue Homogenates, Purified Enzyme Systems	1112	3
Isogenic Fish	1244	95
In-Vivo Alveolar Macrophage Cytotoxicity†	11410	45
In-Vivo Assessment of DNA Damage†	1226	79
In-Vivo Cytogenetics in Mice†	12113	71
In-Vivo DNA Binding†	1228	81
Laboratory Ecosystem	2241	126
LD50	1121	22
Limb Regeneration System	1243	94
Lung Organ Culture System, Tissue Homogenates, Purified Enzyme Systems: Integrated System	1111	2
Lymphocyte Cytotoxicity	1117	10
Maize Waxy Locus Assay	12112	70
Mammalian Platelet and Fibroblast Impairment	1116	9
Mammalian Teratology	1301	100
Marine Algal Assay Bottle Test†	2102	109

(continued)

INDEX II: TEST SYSTEMS IN ALPHABETICAL ORDER (continued)

NAME OF TEST SYSTEM	TEST SYSTEM NUMBER	PAGE
Measurement of Nitrogenase Activity by Acetylene Reduction in Nodulated Plants	2412	143
Metabolic Profiles	1134	29
Metabolism of Chlorinated Hydrocarbons in Subhuman Primates	1133	28
MFO Induction as an Indicator of Toxicity Exposure	1242	93
Mitotic Recombination and Gene Conversion in *Saccaromyces cerevisiae*	1223	76
Model Substrat Metabolism	1135	30
Mouse Lymphoma (L5178Y)	1216	64
Neurobehavioral Toxicological Assessment: Integrated System	1151	48
Oxidant Production by Leukocytes and Alveolar Macrophages Measured by Chemiluminescence	1137	33
Perinatal Toxicology	1302	101
Platelet Secretion Measured by ATP Release	1115	8
Pulmonary Adenoma Bioassay in Mice	1237	90
Pulmonary Function and Pulmonary Metabolism: Integrated System	1145	40
Pulmonary Function in Rats	1146	41
Rabbit Alveolar Macrophage (RAM)	11111	16
Rat Hepatocyte (Liver cell)	11112	18
Pulmonary Function of Unanesthetized Guinea Pigs	1147	42
Reverse-Phase High Pressure Liquid Chromatography (HPLC)	2502	151
Saccharomyces cerevisiae, *Schizosaccharomyces pombe*, Forward and Reverse Mutation	1213	61

(continued)

INDEX II: TEST SYSTEMS IN ALPHABETICAL ORDER (continued)

NAME OF TEST SYSTEM	TEST SYSTEM NUMBER	PAGE
Salmonella typhimurium (Ames)†	1211	58
Sister-Chromatid Exchange Formation (SCE)	1225	78
Sleep-Time Study	1132	27
Soil Core Microcosm	2422	147
Soil/Litter Microcosm	2423	148
Stress Ethylene Bioassay in Plants	2411	142
Subchronic Embryo-Larval, Early Juvenile Fish Toxicity Test	2212	113
Subchronic Embryo-Larval Fish Toxicity Test, Sheepshead Minnow (*Cyprinodon variegatus*)	2312	129
Subchronic Invertebrate Toxicity Test, Chironomid (*Tanytarsus dissimillis*)	2223	120
Subchronic Invertebrate Toxicity Test, Stream Insects	2222	119
Syrian Hamster Embryo Oncogenic Transformation (Focus Assay)	1234	87
Teratology In-Vivo/In-Vitro	1305	104
Terrestrial Microcosm Chamber	2421	146
Tissue Homogenates, Purified Enzyme Systems: Integrated System	1112	3
Toxicity of Aerosolized Pollutants/Acute and Subacute	1141	36
Toxicity of Gaseous and Vapor Phase/Acute and Subacute	1142	37
Tracheal Mucociliary Transport	1114	6
Tradescantia Stamen Hair	12111	69
Tumor Induction in Massive Crustaceans, Molluscs, and Teliost Fish	1241	92
Unscheduled DNA Synthesis (UDS)	1224	77

(continued)

INDEX II: TEST SYSTEMS IN ALPHABETICAL ORDER (continued)

NAME OF TEST SYSTEM	TEST SYSTEM NUMBER	PAGE
Whole Animal LD50 — Oral and Dermal	1122	23
Xenobiotic Mechanisms	1136	32

INDEX III: EXPERIMENTAL SUBJECT LISTING

ORGANISM — SPECIES	TEST TYPE		
	IN-VITRO	IN-VIVO	OTHER
	TEST SYSTEM NUMBER*		
BACTERIA			
Bacillus subtilis	1221		
Escherichia coli	1212 1222		
Salmonella typhimurium	1211 1214 1215		
PLANTS			
Algae	2101 2102		
Duckweed		2231	
Maize		12112	
Plants - general		2411 2412	
Tradescantia		12111	
Yeast	1213 1214 1223	1213 1214 1223	
INSECTS			
Fruit Flies		12110	
Stream Insects		2222	
FISH			
American Flagfish		2213	
Freshwater - general	2502	2211 2212 2214 2215 2501	

(continued)

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*An underlined number indicates that the test system is being applied to more than one organism/species/test type.

INDEX III: EXPERIMENTAL SUBJECT LISTING (continued)

ORGANISM — SPECIES	TEST TYPE IN-VITRO	 IN-VIVO	 OTHER
	TEST SYSTEM NUMBER		
FISH (continued)			
Marine - general	2502	1242 2311 2501	
Sheepshead Minnow		2312 2313	
Teliost		1241	
INVERTEBRATES			
Chironomid		2223	
Crustaceans		1241	
Estuarine Mysid		2324	
Estuarine Shrimp		2323	
Freshwater - general		2221 2501	
Marine - general		2321 2501	
Mollusc		1241	
Water Flea		2224	
MAMMALS			
Chinese Hamster	11110 11113 1217 1218 1219 1225 1229		
Guinea Pig		1145 1147 1303	
Hamster	1233	1303	
Man	1119 1224		
Mouse	1216 1224 1225 1227	121113 1226 1237 1301	

(continued)

INDEX III: EXPERIMENTAL SUBJECT LISTING (continued)

ORGANISM — SPECIES	TEST TYPE		
	IN-VITRO	IN-VIVO	OTHER
	TEST SYSTEM NUMBER		
MAMMALS (continued)			
Mouse	1228 1231 1232 1235 1236	1302 1303 1305	
Rabbit		11111 1137 1138 11410	
Rat	1111 1112 1113 1114 1115 1116 1117 1118 11112	1121 1122 1131 1132 1134 1135 1136 1141 1142 1143 1144 1145 1146 1148 1149 1151 1152 1153 1155 1156 1303 1304 1306	
Subhuman Primates		1133 1153 1154	
Syrian Hamster	1225 1234		
OTHER			
Benthic Assemblage			2322
Estuarine Community			2333

(continued)

INDEX III: EXPERIMENTAL SUBJECT LISTING (continued)

ORGANISM — SPECIES	TEST TYPE		
	IN-VITRO	IN-VIVO	OTHER
		TEST SYSTEM NUMBER	
OTHER (continued)			
Estuarine Microcosmy			2331 2333
Laboratory Ecosystem			2241
Soil Core Microcosm			2422
Soil/Litter Microcosm			2423
Terrestrial Microcosm Chamber			2421

INDEX IV: CONTACT PERSONNEL IN ALPHABETICAL ORDER

CONTACT PERSONNEL	U.S. EPA LABORATORY	TEST SYSTEM NUMBER*
Anderson, R.L.	ERL-DUL	2222, 2223.
Bercz, J.P.	HERL-CIN	1131, 1211, 1213, 1236.
Biesinger, K.E.	ERL-DUL	2221.
Bourquin, A.W.	ERL-GB	2331, 2332.
Brown, M.M.	HERL-RTP	1216.
Brungs, W.A.	ERL-DUL	2212, 2213.
Bull, R.J.	HERL-CIN	1113, 1121, 1131, 1132, 1136, 1155, 1156, 1226, 1236, 1306.
Campbell, K.I.	HERL-CIN	1152.
Carlson, R.	ERL-DUL	2214.
Casciano, D.	NCTR	1211, 1217, 1227.
Chadwick, R.W.	HERL-RTP	1135.
Charles, J.	HERL-RTP	1141, 1142.
Chernoff, N.	HERL-RTP	1303, 1304.
Clarke, N.	HERL-CIN	1215.
Claxton, L.	HERL-RTP	1211, 1212, 1214, 12113, 1221, 1222.
Copeland, M.F.	HERL-RTP	1135.
Couch, J.	ERL-GB	1241.
Courtney, K.D.	HERL-RTP	1301, 1302, 1305.
Daniel, B.	HERL-CIN	1215, 1228.
Drummond, R.	ERL-DUL	2214.
Gardner, D.E.	HERL-RTP	1132, 1136, 1143, 1144, 1149, 11410.

(continued)

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*An underlined number indicates that the test system is used by more than one of the above indicators.

INDEX IV: CONTACT PERSONNEL IN ALPHABETICAL ORDER (continued)

CONTACT PERSONNEL	U.S. EPA LABORATORY	TEST SYSTEM NUMBER*
Garnas, R.L.	ERL-GB	2331, 2332.
Gentile, J.	ERL-NAR	2102.
Gile, J.D.	ERL-COR	2421, 2422.
Gillett, J.W.	ERL-COR	2421.
Graham, J.A.	HERL-RTP	1114, 1117.
Green, J.C.	ERL-COR	2101.
Hansen, D.J.	ERL-GB	2312, 2313.
Hatch, G.	HERL-RTP	1115, 1137, 1138.
Hedtke, S.F.	ERL-DUL, NFTS	2241.
Huisingh, J.L.	HERL-RTP	1119, 11110, 1111, 11112, 11113, 1211, 1212, 1221.
Jackim, E.	ERL-NAR	1229.
Kavlock, R.	HERL-RTP	1303, 1304.
Kowal, N.E.	HERL-CIN	1119, 1219, 1236.
Lazear, E.	NCTR	1211.
Lee, S.D.	HERL-CIN	1111, 1112.
Lighthart, B.	ERL-COR	2423.
Linder, R.	HERL-RTP	1122.
Lingg, R.D.	HERL-CIN	1133, 1134.
Malcolm, A.R.	ERL-NAR	1218, 1225, 1229.
McCabe, L.J.	HERL-CIN	1211, 12111.
McKim, J.M.	ERL-DUL	2212.
Miller, W.E.	ERL-COR	2101.
Moore, W.	HERL-CIN	1143, 1144.
Nesnow, S.	HERL-RTP	1134, 1231, 1232, 1234, 1235.
Nimmo, D.W.	ERL-GB	2324.
O'Neil, J.J.	HERL-RTP	1145.
Orthoefer, J.	HERL-CIN	1237.
Pahren, H.	HERL-CIN	1116, 1118, 1211, 1213, 1233.

(continued)

INDEX IV: CONTACT PERSONNEL IN ALPHABETICAL ORDER (continued)

CONTACT PERSONNEL	U.S. EPA LABORATORY	TEST SYSTEM NUMBER*
Pepelko, W.E.	HERL-CIN	1146, 1148.
Pesch, G.G.	ERL-NAR	1225.
Pritchard, P.H.	ERL-GB	2331, 2332.
Reiter, L.	HERL-RTP	1151, 1153, 1154.
Richards, N.	ERL-GB	1211, 1242, 1243, 1244, 1245, 1246.
Robinson, E.	ERL-DUL, NFTS	2101.
Sandhu, S.S.	HERL-RTP	1212, 1213, 1214, 1216, 1217, 1219, 12110, 12111, 12112, 12113, 1221, 1223, 1225.
Schimmel, S.C.	ERL-GB	2501.
Schoor, P.	ERL-GB	1242.
Shiroyama, T.	ERL-COR	2101.
Specht, D.	ERL-COR	2102.
Spehar, R.	ERL-DUL	2213.
Spoor, W.A.	ERL-GB	2215.
Stara, J.F.	HERL-CIN	1211, 1214, 12113.
Stephan, C.	ERL-DUL	2211, 2221, 2224, 2311, 2321.
Swartz, R.	ERL-COR	2322.
Tagatz, M.	ERL-GB	2333.
Tingey, D.T.	ERL-COR	2411.
Tyler-Schroeder, D.B.	ERL-GB	2323.
Veith, G.D.	ERL-DUL	2501, 2502.
Walsch, G.	ERL-GB	2102.
Waters, M.D.	HERL-RTP	1119, 11111, 1211, 1212, 1213, 1214, 1216, 1217, 1219, 12110, 12111, 12112, 12113, 1221, 1222, 1223, 1224, 1225, 1234.
Wickliff, C.	ERL-COR	2412.
Wiester, M.J.	HERL-CIN	1147.

INDEX V: U.S. EPA LABORATORIES IN ALPHABETICAL ORDER

U.S. EPA LABORATORY	TEST SYSTEM NUMBER*
Environmental Research Laboratory-Corvallis, Oregon	2101, 2102, 2322, 2411, 2412, 2421, 2422, 2423.
Environmental Research Laboratory-Duluth, Minnesota	2101, 2211, 2212, 2213, 2214, 2215, 2221, 2222, 2223, 2224, 2231, 2241, 2311, 2321, 2501, 2502.
Environmental Research Laboratory-Gulf Breeze, Florida	1211, 1241, 1242, 1243, 1244, 1245, 1246, 2102, 2312, 2313, 2323, 2324, 2331, 2332, 2333, 2501.
Environmental Research Laboratory-Narragansett, Rhode Island	1218, 1225, 1229, 2102.
Health Effects Research Laboratory-Cincinnati, Ohio	1111, 1112, 1113, 1116, 1118, 1119, 1121, 1131, 1132, 1133, 1134, 1136, 1143, 1144, 1146, 1147, 1148, 1152, 1155, 1156, 1211, 1213, 1214, 1215, 1219, 12111, 12113, 1226, 1228, 1233, 1236, 1238, 1306.
Health Effects Research Laboratory-Research Triangle Park, North Carolina	1114, 1115, 1117, 1119, 11110, 11111, 11112, 11113, 1122, 1132, 1134, 1135, 1136 1137, 1138, 1141, 1142, 1143, 1144, 1145, 1149, 11410, 1151, 1153, 1154, 1211, 1212, 1213, 1214, 1216, 1217, 1219, 12110, 12111, 12112, 12113, 1221, 1222, 1223, 1224, 1225 1227, 1231, 1232, 1234, 1235, 1301, 1302, 1303, 1304, 1304.
National Center for Toxicological Research-Jefferson, Arkansas	1211, 1217, 1227.

*An underlined number indicates that the test system is used by more than one laboratory.

INDEX VI: U.S. EPA DEPARTMENTAL LISTING IN ALPHABETICAL ORDER

DEPARTMENT	U.S. EPA LABORATORY	CONTACT PERSONNEL
	ENVIRONMENTAL RESEARCH LABORATORY-CORVALLIS 200 SW 35th Street Corvallis, Oregon 97330	
Assessment Criteria Division		
Special Studies Branch		J.C. Green W.E. Miller T. Shiroyama
Ecological Effects Research Division		
Marine and Freshwater Branch		D. Specht
Newport Field Station		R. Swartz
Terrestrial Ecology Branch		S. Draggon J.D. Gile J.W. Gillett B. Lighthart D.T. Tingey C. Wickliff
	ENVIRONMENTAL RESEARCH LABORATORY-DULUTH 6201 Congdon Boulevard Duluth, Minnesota 55803	
Extramural Program Branch		K.E. Biesinger
Newtown Fish Toxicology Station		S.F. Hedtke E. Robinson
Research Branch		
Chemical Pollutant Section		G.D. Veith
Physical Pollutant Section		R.L. Anderson
Physical Effects of Toxicants Section		W.A. Brungs R. Carlson R. Drummond J.M. McKim R. Spehar

(continued)

INDEX VI: U.S. EPA DEPARTMENTAL LISTING IN ALPHABETICAL ORDER (continued)

DEPARTMENT	U.S. EPA LABORATORY	CONTACT PERSONNEL
	ENVIRONMENTAL RESEARCH LABORATORY-DULUTH (continued)	
Technical Assistance Branch		C. Stephan
	ENVIRONMENTAL RESEARCH LABORATORY-GULF BREEZE Sabine Island Gulf Breeze, Florida 32561	
Experimental Environments Branch		D.J. Hansen D.W. Nimmo S.C. Schimmel M. Tagatz D.B. Tyler-Schroeder C. Walsch
Office of the Director (Carcinogenic Research Team)		J. Couch N. Richards W.A. Spoor
Processes and Effects Branch		A.W. Bourquin R.L. Garnas P.H. Pritchard
	ENVIRONMENTAL RESEARCH LABORATORY-NARRANGANSETT South Ferry Road Narrangansett, Rhode Island 02882	
Toxicology Branch Genetic Toxicology Team		E. Jackim A.R. Malcolm G.G. Pesch
Marine Toxicology Team		J. Gentile
	HEALTH EFFECTS RESEARCH LABORATORY-CINCINNATI 26 West St. Clair Street Cincinnati, Ohio 45268	
Field Studies Division Toxicological Assessment Branch		J.P. Bercz N.E. Kowal L.J. McCabe H. Pahren J.F. Stara

(continued)

INDEX VI: U.S. EPA DEPARTMENTAL LISTING IN ALPHABETICAL ORDER (continued)

DEPARTMENT	U.S. EPA LABORATORY	CONTACT PERSONNEL
	HEALTH EFFECTS RESEARCH LABORATORY-CINCINNATI (continued)	
Laboratory Studies Division		
Exposure Evaluation Branch		R.D. Lingg
Functional Pathology Branch		S.D. Lee J. Orthoefer W. Moore W.E. Pepelko M.J. Wiester
Toxicological Assessment Branch		R.J. Bull K.I. Campbell N. Clarke B. Daniel
	HEALTH EFFECTS RESEARCH LABORATORY-RESEARCH TRIANGLE PARK Research Triangle Park North Carolina 27711	
Clinical Studies Division		
Biomedical Research Branch		D.E. Gardner J.A. Graham G. Hatch J.J. O'Neil
Experimental Biology Division		
Developmental Biology Branch		N. Chernoff R. Kavlock
Neurobiology Branch		L. Reiter
Environmental Toxicology Division		
Biochemistry Branch		M.M. Brown R.W. Chadwick L. Claxton M.F. Copeland J.L. Huisingh S. Nesnow S.S. Sandhu M.D. Waters

(continued)

INDEX VI: U.S. EPA DEPARTMENTAL LISTING IN ALPHABETICAL ORDER (continued)

DEPARTMENT	U.S. EPA LABORATORY	CONTACT PERSONNEL
	HEALTH EFFECTS RESEARCH LABORATORY-RESEARCH TRIANGLE PARK (continued)	
Toxic Effects Branch		J. Charles K.D. Courtney R. Linder
	NATIONAL CENTER FOR TOXICOLOGICAL RESEARCH Jefferson, Arkansas 72079	
Division of Mutagenesis Research Somatic Cell Section		D.A. Casciano E. Lazear

INDEX VII: GRANT/CONTRACT LABORATORIES IN ALPHABETICAL ORDER

GRANT/CONTRACT LABORATORY	PRINCIPAL INVESTIGATOR	TEST SYSTEM NUMBER
American Health Foundation Naylor Dana Institute for Disease Prevention Hammond House Road Valhalla, New York 10595	G.M. Williams	1227.
Ball State University Muncie, Indiana 47306	D. Adalis	1114.
Bionomics EG & G Route 6, Box 1002 Pensacola, Florida 32507	P.R. Parrish	2501, 2312, 2313, 2324.
Bionomics, Inc. Wareham, Massachusetts	K.S. Macek	2211, 2212, 2221, 2311, 2321.
	S. Sauter	2211, 2212, 2221 2311, 2321.
Brookhaven National Laboratories Long Island, New York	L. Shirer	12111.
California, University of Davis, California 95616	E. Goldstein	11410.
California, University of School of Medicine Los Angeles, California 90032	M.G. Mustafa	1112.
California, University of Medical Center San Francisco, California 94132	R.S. Bhatnagar	1111.
Cincinnati, University of Cincinnati, Ohio 45221	C. Smith	1133.
Cincinnati University of Medical Center Cincinnati, Ohio 43221	J.C. Loper D. Lang	1211, 1236. 1236.

(continued)

GRANT/CONTRACT LABORATORY	PRINCIPAL INVESTIGATOR	TEST SYSTEM NUMBER
Colorado, University of Medical Center 4200 East 9th Avenue, Denver Colorado 80262	C.C. Solomons	1116.
	W.L. Weston	1118.
Denver Research Institute Denver, Colorado 80210	J. Schmidt-Coderis	1246.
Florida State University Tallahassee, Florida 32306	B. Glasson	2333.
Georgia State University Atlanta, Georgia 30303	D.G. Ahearn	2332.
Gulf Coast Research Laboratory P.O. Box Drawer AG Ocean Springs, Mississippi 39564	W.W. Walker	2332.
Gulf South Research Institute P.O. Box 26518 New Orleans, Louisiana	N. Gruener	1119, 1219, 1236.
	E. Kline	1245.
IIT Research Institute 10 West 35th Street Chicago, Illinois 60616	C. Aranyi	11111, 11410.
	R. Ehrlich	1149.
	L. Schiff	1114.
Illinois, University of Urbana, Illinois 61801	M. Plewa	12112.
Iowa State University Ames, Iowa 50010	W.E. Lloyde	1153, 1154.
Litton Biometics, Inc. Nicholson Lane Kensington, Maryland	D.T. Brusick	1211.
Louisiana State University Medical School New Orleans, Louisiana 70112	W. Pelon	1211.
Medical College of Virginia Richmond, Virginia 23298	R.L. Balster	1152.
	J.F. Borzelleca	1152.
	W.L. Dewey	1152.
	A.E. Munson	1152.

(continued)

INDEX VII: GRANT/CONTRACT LABORATORIES IN ALPHABETICAL ORDER (continued)

GRANT/CONTRACT LABORATORY	PRINCIPAL INVESTIGATOR	TEST SYSTEM NUMBER
Michigan, University of Ann Anbor, Michigan 48104	A. Beaudoin	1305.
	E. Goodman	2421.
Microbiological Associates Bethesda, Maryland 20014	R. Kouri	1231, 1235, 1237.
	L. Schectman	1231, 1235, 1237.
Missouri, University of Columbia, Missouri 65201	C. Marianseld	1211.
	J.T. O'Conner	12111.
Norih Carolina, University of Chapel Hill, North Carolina 27514	A. Collier	1114.
	D. Humm	1244.
Northrop Services, Inc. P.O. Box 12313 Research Triangle Park, North Carolina 27709	B. Adkins	1114, 1149.
	N.E. Garrett	1119, 11110, 11111, 11113.
Oak Ridge National Laboratory P.O. Box Y Oak Ridge, Tennessee 37830	A. Hsie	1217.
Ohio State University Columbus, Ohio 43210	R.W. Hart	1226.
Oregon, University of Eugene, Oregon 97403	M. Mix	1241.
Rockefeller University 1230 York Avenue New York, New York 10021	M. Bowers	1117.
Southern Mississippi, University of Hattiesburg, Mississippi 39401	B.J. Martin	1241.
Southwest Research Institute San Antonio, Texas 78284	E. Gause	11410.
Stanford Research Institute Menlo Park, California 94025	A. Mitchell	1216, 1219, 12110, 1224.
	G. Newell	12111, 12113.
	V.F. Simmons	1211, 1212, 1213, 1214, 1221, 1222, 1223, 1225.

(continued)

INDEX VII: GRANT/CONTRACT LABORATORIES IN ALPHABETICAL ORDER (continued)

GRANT/CONTRACT LABORATORY	PRINCIPAL INVESTIGATOR	TEST SYSTEM NUMBER
Syracuse Research Corporation Merrill Lane Syracuse, New York 13210	J. Saxena	1233.
University of Texas Medical Branch Galveston, Texas 77550	M. Legator	1211, 1214, 12113.
University of West Florida Pensacola, Florida 32504	J. Bazlis	1211.
	R. Rao	1242, 1243, 1245.
Wisconsin, University of Madison, Wisconsin 53706	P. Lichtenstein	2421.

INDEX VIII: STATUS OF DEVELOPMENT DISTRIBUTION OF THE TEST SYSTEMS

STATUS OF DEVELOPMENT	TEST SYSTEM NUMBER
Developmental:	1115, 1116, 1117, 1118, 11112, 11113, 1153, 1154, 1215, 1218, 12111, 12112, 1226, 1227, 1228, 1231, 1233, 1234, 1242, 1244, 1245, 1246, 1304, 2215, 2222, 2223, 2231, 2241, 2322, 2323, 2331, 2333, 2421, 2322, 2423.
Being implemented:	1111, 1119, 11110, 11111, 11112, 1122, 1134, 1135, 1137, 1138, 1141, 1142, 1145, 1147, 11410, 1151, 1152, 1155, 1214, 1216, 1217, 1219, 1223, 1225, 1229, 1232, 1234, 1236, 1237, 1241, 1243, 1305, 1307, 2214, 2224, 2312, 2313, 2324, 2332, 2411, 2412, 2501, 2502.
Validated:	1112, 1113, 1114, 1121, 1131, 1132, 1133, 1136, 1143, 1144, 1146, 1148, 1149, 14110, 1156, 1211, 1212, 1213, 12110, 12113, 1221, 1222, 1224, 1301, 1302, 1303, 2101, 2102, 2211, 2212, 2213, 2221, 2311, 2321.

INDEX IX: ENDPOINTS OF THE GENERAL AND PERINATAL TOXICITY TEST SYSTEMS

ENDPOINTS	TEST SYSTEM NUMBER
Adrenal function	1131.
Airway resistence	1145, 1147.
Alveolar macrophage - bacterial activity	1137.
Alveolar macrophage - cytotoxicity	11410.
Alveolar macrophage - enzymatic profile	11410.
Alveolar macrophage - morphology	11410.
Arterial blood P_{CO_2}	1148.
Arterial blood P_{O_2}	1148.
ATP, ADP, AMP tissue levels	1113, 1116, 11111, 11112, 1156, 1306.
ATP release from platelets	1115.
Bicarbonate concentration in the blood	1148.
Biochemical development of the nervous system	1156, 1306.
Blood pH	1148.
Brain neurochemistry	1152, 1156.
Breathing frequency	1145, 1147.
cAMP	1138.
Cardio-vascular response	1147.
Cell number	1119, 11111.
cGMP	1138.

(continued)

INDEX IX: ENDPOINTS OF THE GENERAL AND PERINATAL TOXICITY TEST SYSTEMS (continued)

ENDPOINTS	TEST SYSTEM NUMBER
Ciliary beating	1114.
Clinical symptoms	1121, 1122.
Colony formation	11110, 11113.
Comparative metabolism	1133, 1134, 1135.
Creative phosphokinase	1302.
Cytological	1114, 11410.
Cytochrome P-450	1136, 1156, 1306.
CNS Function	1151, 1152, 1156.
Deposition	1114, 1144.
Dose-response curve	1122, 1141, 1142, 1143, 1145.
Electrocardiograms (ECG)	1147.
Enzyme activity	1111, 1112, 1132, 1133, 1142, 1145, 11410.
Fetal anomalies	1301, 1303, 1304, 1305, 1306.
Fetal malformation	1301, 1303.
Fetal toxicity	1301, 1302, 1303, 1304, 1305, 1306.
Growth of neonatal rats	1304.
Heart-rate	1147.
Hematology	1152.
Histology	1114, 1141, 1142.
Hydrolytic enzyme activity	11110.
Immune response and host resistance	1152.

(continued)

INDEX IX: ENDPOINTS OF THE GENERAL AND PERINATAL TOXICITY TEST SYSTEMS (continued)

ENDPOINTS	TEST SYSTEM NUMBER
Infectivity model	1149.
Isozyme profiles	1131, 1302.
LC50	1141, 1142, 1143.
LD50	1121, 1122.
Learning behavior in rats	1151, 1152.
Learning in primates	1151, 1153, 1154.
Lethality	1141, 1142, 1143, 1149, 1152, 1301, 1303, 1304, 1305, 1306.
Liver enzymes	1136.
Locomotor activity	1151, 1152, 1153, 1304.
Lung clearance	1144.
Lung compliance	1145, 1147.
Lymphocyte cytotoxicity	1117.
Mammalian teratology	1301.
Maximum tolerated dose (MTD)	1136.
1/2 MTD	1136.
1/4MTD	1136.
Metabolites	1111, 1112, 1113, 1133, 1134, 1135, 1136, 1142.
Memory in primates	1151, 1153, 1154.
Minute volumes	1145, 1147.
Morphology	1119, 11111, 11112.
Motor and sensory activity	1151, 1152, 1153, 1154.

(continued)

INDEX IX: ENDPOINTS OF THE GENERAL AND PERINATAL TOXICITY TEST SYSTEMS (continued)

ENDPOINTS	TEST SYSTEM NUMBER
Mutation frequency	11113.
NADH	1113, 1156.
NADPH	1113, 1156.
Neutrophil phagocytosis	1118.
Oxidatant production in alveolar macrophages	1137.
Phagocytic index	1118. 11111.
Platelet function	1115, 1116.
Post natal development	1302, 1304, 1305, 1306.
Protein determinations	1119, 1305.
Pulmonary mechanics	1145, 1146, 1147.
Residual lung volumes	1145, 1146, 1147.
Righting reflex	1132.
Sensory modality	1151, 1152, 1153.
Sequencing of behavior	1151, 1152, 1153.
Serum constituents	1131.
Serum isoenzyme patterns	1131, 1302.
Sleep-time	1132.
Somite development	1305.
Static compliance curves	1145, 1146, 1147.
Task discrimination	1151, 1154.
Teratogenicity	1301, 1302, 1303, 1304, 1305, 1306.
Thyroid function	1131.

(continued)

INDEX IX: ENDPOINTS OF THE GENERAL AND PERINATAL TOXICITY TEST SYSTEMS (continued)

ENDPOINTS	TEST SYSTEM NUMBER
Tidal volume	1145, 1147.
Total cell protein	1119, 11111, 11112.
Urinary constituents	1131.
Viability of neonatal rats	1304, 1306.

INDEX X: ENDPOINT DISTRIBUTION OF THE GENOTOXICITY TEST SYSTEMS

ENDPOINTS	TEST TYPE		
	IN-VITRO	IN-VIVO	OTHER
	TEST SYSTEM NUMBER*		
Carcinogenesis	1231 1232 1233 1234 1235 1236	1237 1241 1242 1246	
Mutagenesis			
Chromosomal aberrations	1225	12113 1225	1218 1219 12110
Point mutation	1211 1212 1214 1216 1217 1218 1219	1213 1214 12110 12111 12112 1242 1245	
Primary DNA Damage	1215 1218 1219 1221 1222 1224 1227 1229	1223 1226 1228 1242	1245

*An underlined number indicates that the test system has more than one endpoint.

INDEX XI: ENDPOINT DISTRIBUTION OF THE ECOLOGICAL TEST SYSTEMS

ENDPOINT	TEST SYSTEM NUMBER*
Bioconcentration Factors (BCF)	2501, 2502.
Effect Concentration (EC)	2101, 2102, 2212, 2214, 2215, 2223, 2224, 2231, 2312, 2313, 2322, 2331, 2332, 2333, 2411, 2412, 2421, 2422, 2423.
50% Effect Concentration (EC50)	2221, 2222, 2321.
50% Lethal Concentration (LC50)	2211, 2221, 2222, 2223, 2224, 2311, 2312, 2321, 2323, 2324.
Maximum Acceptable Toxicant Concentration (MATC)	2212, 2213, 2241†, 2312.

*An underlined number indicates that the test system has more than one endpoint.

†This endpoint is based on change within the ecosystem processes.

INDEX XII: APPLICATION DISTRIBUTION TABLE

APPLICATION:	AIR	WATER	SOIL	FOOD	MULTIMEDIA
TEST SYSTEM NUMBER:					1111
					1112
					1113
	1114				
	1115	1115		1115	
		1116			
					1117
		1118			
	1119	1119			
	11110	11110			
	11111	11111			
					11112
	11113	11113			
					1121
					1122
					1131
					1132
					1133
					1134
					1135
					1136
					1137
	1138	1138		1138	1138
	1141				
	1142				
					1143
					1144
	1145				
	1146	1146		1146	
	1147				1147
	1148	1148		1148	
	1149				
	11410				
					1151
		1152			
					1153
					1154
		1155			

(continued)

INDEX XII: APPLICATION DISTRIBUTION TABLE (continued)

APPLICATION:	AIR	WATER	SOIL	FOOD	MULTIMEDIA
TEST SYSTEM NUMBER:					1156
					1211
					1212
					1213
					1214
					1215
					1216
					1217
					1218
					1219
					12110
	12111	12111			
	12112		12112		12112
					12113
					1221
					1222
					1223
					1224
					1225
					1226
					1227
					1228
		1229			1229
	1231				
					1232
		1233			
	1234				
	1235				
		1236			
					1237
		1241			
		1242			
		1243			
		1244			
		1245		1245	
		1246			1246
					1301
					1302
					1303
	1304	1304		1304	
					1305
					1306
		2101			
		2102			
		2211			

(continued)

INDEX XII: APPLICATION DISTRIBUTION TABLE (continued)

APPLICATION:	AIR	WATER	SOIL	FOOD	MULTIMEDIA
TEST SYSTEM NUMBER:		2212			
		2213			
		2214			
		2215			
		2221			
		2222			
		2223			
		2224			
		2231			
		2241			
		2311			
		2312			
		2313			
		2321			
		2322	2322		
		2323			
		2324			
		2331			
		2332			
		2333			
	2411				
			2412		
	2421	2421	2421		
			2422		
			2423		
		2501			
		2502			

INDEX XIII: SAMPLE DISTRIBUTION OF THE GENERAL AND PERINATAL TEST SYSTEMS

SAMPLES	TEST SYSTEM NUMBER
Aerosols	1141.
Air	1113, 1114, 1115, 1141, 1142, 1145, 1146, 1147, 1148, 1149, 11410.
Alkylating agents	11113.
Aromatic amines	11113.
Asbestos	1146.
AWT effluent	1119.
Benzene	1152.
Cadmium	1111, 1112, 1114, 11111, 1149, 11410.
Chlorinated aliphatic hydrocarbons	1133, 1134.
Chlorinated aromatic hydrocarbons	1133, 1134.
Coal dust	1146, 1148.
Copper	1111.
Diesel fuel	1111, 1113, 1145, 1146, 1147, 1148.
Dioxin	1152, 1301.
Dolomite	11110.
Drugs	1135.

(continued)

INDEX XIII: SAMPLE DISTRIBUTION OF THE GENERAL AND PERINATAL TOXICITY TEST SYSTEMS (continued)

SAMPLES	TEST SYSTEM NUMBER
Energy related pollutants	1113, 1114, 1115, 1117, 11110, 11111, 1132, 1138, 1145, 1146, 1149, 11410, 1155, 1156, 1306.
Ethers	1152.
Fly ash	1119, 11110, 11111.
Gases	1111, 1145, 1149, 11410.
Gasoline exhaust	1113, 1114, 1115, 1146.
Heavy metals	1111, 1119, 11113, 1146. 1151, 1154, 1303, 1306.
Hydrocarbons	1116, 1118. 11113.
Industrial pollutants	1113, 1114, 1115, 1117, 1119, 11110, 11111, 1132, 1138, 1141, 1142, 1146, 1148, 1149, 11410, 1155, 1156, 1306.
Inorganics	1119, 11112, 1301, 1302, 1303, 1304, 1306.
Manganese	1111, 11111, 11410.
Mercury	1111, 1112.
Nickel	1114, 11111, 1149, 11410.
Nitrogen oxides	1111, 1138, 1145.
Nitroso amines	11113.
NO	1138.
NO_2	1111, 1112, 1137, 1138, 1145, 1149, 11410.
Non-ionizing radiation	1151.

(continued)

INDEX XIII: SAMPLE DISTRIBUTION OF THE GENERAL AND PERINATAL TOXICITY TEST SYSTEMS (continued)

SAMPLES	TEST SYSTEM NUMBER
Organics	1116, 1119, 11112, 1121, 1122, 1131, 1145, 1155, 1301, 1302, 1303, 1304, 1305, 1306.
Oxidants	1112, 1146, 1148.
Ozone	1112, 1137, 1138, 1145, 1148, 1149, 11410.
Paraquat	1145, 1148.
Particulates	11110, 1137, 1141, 1145, 1149, 11410.
Pesticides	1122, 1135, 1141, 1142 1151, 1152, 1301, 1302, 1303, 1304, 1305.
Phenyls	1152.
Platium	11111.
Radioactive aerosols	1144.
Rock dust	1148.
Silicic acid	11110.
SO_2	1111, 1112, 1145, 1147, 1148.
Stack gases	1146, 1148.
Sulfates	1145, 1146, 1148, 1149.
Sulfuric acid	1114, 1145.
Sulfur oxides	1111, 1145.
Technical grade materials	1122, 1141, 1142, 1143.

(continued)

INDEX XIII: SAMPLE DISTRIBUTION OF THE GENERAL AND PERINATAL TOXICITY TEST SYSTEMS (continued)

SAMPLES	TEST SYSTEM NUMBER
Toxic substances	11110, 1121, 1122, 1131, 1132, 1135, 1141, 1142, 1143, 1145, 1151, 1152, 1301, 1302, 1303, 1304, 1305.
Trace metals	1111, 1119, 11111, 1148, 11410, 1151, 1155, 1303, 1306.
Transportation related pollutants	1113, 1114, 1115, 1117, 1138, 1145, 1146, 1147, 1148, 1149, 11410, 1155, 1156, 1306.
Trihalomethanes	1152.
Vaporizable liquids	1142.
Water	1115, 1116.
Xenobiotics	1135, 1136.
Zinc	1111.

INDEX XIV: SAMPLE DISTRIBUTION OF THE GENOTOXICITY TEST SYSTEMS*†

SAMPLES	TEST SYSTEM NUMBER
ALKYLATING AGENTS	
Aldehydes	1211, 1212, 1213, 1216, 1217, 1218, 1219, 12110.
Alkane sulfonic esters	1211, 1212, 1213, 1216, 1217, 1218, 1219, 12110, 12111, 12113, 1221, 1222, 1223, 1224.
Alkyl and alkane halides	1211, 1212, 1213, 1216, 1217, 1218, 1219, 12110, 12111, 1221, 1222, 1223.
Alkylsulfates	1211, 1212, 1213, 1216, 1217, 1218, 1219, 12110, 1221, 1222, 1223.
Aryldialkyltriazenes	1212, 12110, 12111.
Aziridines	1211, 1212, 1213, 1216, 1217, 1218, 1219, 12110, 12111, 12113, 1221, 1222, 1223.
Azoxy and hydrazo alkanes	1211, 12110, 1224.
Diazoalkanes	1211, 1212, 1213, 1216, 1217, 1218, 1219, 12110.
Epoxides	1211, 1212, 1213, 1216, 1217, 1218, 1219, 12110, 12111, 12113, 1221, 1222, 1223.
Lactones	1211, 1212, 1213, 1216, 1217, 1218, 1219, 12110, 1221, 1222, 1223, 1224,
Nitrogen, sulfur, and oxide mustards	1211, 1212, 1213, 1216, 1217, 1218, 1219, 12110, 12113, 1221, 1222, 1223, 1224.
Phosphoric acid esters	1211, 1212, 1216, 1217, 1218, 1219, 12110, 12111, 1221, 1222, 1223.
Sultones	1211, 1212, 1213, 1221, 1222, 1223, 1224.

(continued)

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*The data base on test systems 1214, 1215, and 12112 is not yet available.
†The scope of this index extends beyond the test system texts.

INDEX XIV: SAMPLE DISTRIBUTION OF THE GENOTOXICITY TEST SYSTEMS (continued)

SAMPLES	TEST SYSTEM NUMBER
ALKYLATING AGENTS (continued)	
Triazines	1211, 1212, 1213, 1216, 1217, 1218, 1219, 12110, 12111, 12113, 1224.
AROMATIC AMIDES	1211, 1212, 1213, 1216, 1217, 1218, 1219, 12110, 12111, 12113, 1224.
AZO DYES	1211, 1212, 1216, 1217, 1218, 1219, 12110, 1224.
HALOGENATED ETHERS AND HALOHYDRINS	1211, 1212, 1216, 1217, 1218, 1219, 12110, 12111.
HALOGENATED HYDROCARBONS AND RELATED DERIVATIVES	
Fluorocarbons	1211, 1212, 1213, 12110.
Halogenated aromatics	1211, 1212, 12110, 12111.
Vinyl and vinylidine derivatives	1211, 1212, 1216, 1218, 1219, 12110.
HETEROCYCLICS	
Acridines and quinicrines	1211, 1212, 1213, 1216, 1217, 1218, 1219, 12110, 1221, 1222, 1223, 1224.
Benzimidazoles	1211, 1212, 12110.
Cyclodienes	1211, 1212.
Dibenzo-p-dioxins	1211, 1212.
Dicarboximides	1211, 1212.
Fluorenones	1211, 1212, 1216, 1217, 1218, 1219, 12110, 1221, 1222, 1223, 1224.
Fuorocoumarins	1211, 1212.
Phenothiazines	1211, 1212.
Thioxanthines	1211, 1212, 1213, 1216, 1217, 1218, 1219.
Other	1211, 1212, 1213, 1216, 1217, 1218, 1219, 12110, 12111.

(continued)

INDEX XIV: SAMPLE DISTRIBUTION OF THE GENOTOXICITY TEST SYSTEMS (continued)

SAMPLES	TEST SYSTEM NUMBER
HYDRAZINES, HYDROXYLAMINES CARBAMATES, HYDRAZIDES, AND UREAS	
Carbamates	1211, 1212, 1213, 1216, 1217, 1218, 1219, 12110, 12111, 1221, 1222, 1223.
Hydrazides	1211, 1212, 12111.
Hydrazines	1211, 1212, 12110.
Hydroxylamines	1211, 1212, 1213, 1216, 1217, 1218, 1219, 12110, 12111, 1221, 1222, 1223.
Ureas and thioureas	1211, 1212, 1213, 1216, 1217, 1218, 1219, 12110, 12111.
INORGANIC DERIVATIVES	
Halogens and derivatives	1211, 1212, 1216, 1217, 1218, 1219, 12110.
Metal and metalloid derivatives	1211, 1212, 1213, 1216, 1217, 1218, 1219, 12110, 12111, 1221, 1222, 1223.
Ozone	1212, 12110, 12111.
Sulfur and nitrogen oxides	1211, 1212, 1213, 12110, 12111, 1221, 1222, 1223.
NATURAL PRODUCTS	
Antibotics	1211, 1212, 1213, 1216, 1217, 1218, 1219, 12110, 1221, 1222, 1223, 1224.
Mycotoxins	
Aflatoxin	1211, 1212, 1216, 1217, 1218, 1219, 12110, 1221, 1222, 1223, 1224.
Other	1211, 1212, 12110, 1221, 1222, 1223, 1224.
Pyrrolizidine alkaloids	12110, 1221, 1222, 1223.
Steroids	1211, 1212, 1213, 1216, 1217, 1218, 1219, 12110.
Xanthines	1211, 1212, 1213, 1216, 1217, 1218, 1219, 12110, 12111, 1221, 1222, 1223, 1224.
NITRO DERIVATIVES	1211, 1212, 1213, 1216, 1217, 1218, 1219, 12110, 12111.

(continued)

INDEX XIV: SAMPLE DISTRIBUTION OF THE GENOTOXICITY TEST SYSTEMS (continued)

SAMPLES	TEST SYSTEM NUMBER
NITRO DERIVATIVES (continued)	
Nitrofurans	1211, 1212, 1216, 1217, 1218, 1219, 12110, 1221, 1222, 1223.
Nitroimidazoles	1212, 12110.
Nitroquinolines and compounds	1211, 1212, 1213, 12110, 1221, 1222, 1223, 1224.
N-NITROSO COMPOUNDS	
Nitrosamides	1211, 1212, 1213, 1216, 1217, 1218, 1219, 12110, 12111, 1221, 1222, 1223, 1224.
Nitrosamines	1211, 1212, 1213, 1216, 1217, 1218, 1219, 12110, 12111, 1221, 1222, 1223, 1224.
Nitrosoureas	1211, 1212, 1213, 1216, 1217, 1218, 1219, 12110.
NUCLEIC ACID BASES AND ANALOGS	1211, 1212, 1213, 1216, 1217, 1218, 1219, 12110, 12111, 12113.
ORGANIC PEROXIDES	1211, 1212, 12110.
ORGANO-METALLICS	
Organo lead derivatives	1221, 1222, 1223.
Organo mercury derivatives	1211, 1212, 12111, 1221, 1222, 1223.
Other	1211, 1212, 12110, 1221, 1222, 1223.
POLYNUCLEAR AROMATICS	1211, 1212, 1216, 1217, 1218, 1219, 12110, 1221, 1222, 1223, 1224.
OTHER	
Esters and anhydrides	1211, 1212, 1213, 12110, 12111.
N-oxides	1211, 1212, 1213, 12110.
Quaternary ammonium compounds	1211, 1212, 1213, 1216, 1217, 1218, 1219, 12110.
Quinones	1211, 1212, 1216, 1217, 1218, 1219, 12110, 12111.
Sulfites	1212, 1213, 12110, 12111.

INDEX XV: SAMPLE DISTRIBUTION OF THE ECOLOGICAL TEST SYSTEMS

SAMPLES	TEST SYSTEM NUMBER
Acid rain	2411.
Air pollutants	2411, 2421.
Ambient	2101, 2102, 2411, 2423.
Ambient air	2411.
Chloramines	2102.
Chlorinated hydrocarbons	2501.
Chlorine	2102.
CO	2411.
Coal storage	2101.
Complex wastes	2322.
Detergents	2101.
Dissolved gases	2215.
Dredge sediment	2102, 2322.
Drilling muds	2211, 2221, 2311, 2321, 2333.
Energy related	2101, 2211, 2212, 2215, 2221, 2224, 2231, 2311, 2321, 2331, 2332, 2333, 2411, 2423.
Gaseous pollutants	2411, 2421.
Heavy metals	2101, 2102, 2211, 2221, 2222, 2223, 2311, 2321, 2322, 2332, 2412, 2421, 2422, 2423, 2501.

(continued)

INDEX XV: SAMPLE DISTRIBUTION OF THE ECOLOGICAL TEST SYSTEMS (continued)

SAMPLES	TEST SYSTEM NUMBER
Herbicides	2101, 2211, 2221, 2311, 2312, 2313. 2321.
Industrial	2101, 2102, 2211, 2212, 2214, 2215, 2221, 2224, 2231, 2311, 2321, 2331, 2332, 2411, 2412, 2421, 2422, 2423, 2502.
Industrial air pollutants	2411.
Industrial effluents	2211, 2221, 2311, 2321, 2331, 2332, 2421.
Industrial sludge	2412.
Industrial waste discharges	2101.
Industrial waste effluents	2422.
Inorganics	2212, 2241, 2502.
Insecticides	2101, 2211, 2221, 2311, 2312, 2313. 2321, 2501.
Leachates	2101.
Metals	2213, 2215, 2224, 2231, 2324.
Multi chemicals	2214.
Monochlorinated organics	2102.
NH_3	2101.
NO_2	2101.
NO_3	2101.
NO_x	2411.
Nutrients	2101, 2102.

(continued)

INDEX XV: SAMPLE DISTRIBUTION OF THE ECOLOGICAL TEST SYSTEMS (continued)

SAMPLES	TEST SYSTEM NUMBER
Oils	2211, 2221, 2311, 2321, 2331, 2332.
Organics	2212, 2215, 2224, 2231, 2241, 2331, 2332, 2502.
Ortho-P	2101.
Other	2101.
Oxygen	2215.
Ozone	2411.
PCB's	2102, 2211, 2221, 2311, 2312, 2321, 2501.
Pentachlorophenol	2312.
Pesticides	2101, 2213, 2222, 2323, 2324, 2331, 2332, 2333, 2421, 2422, 2423.
Radiolabeled material	2501.
Receiving waters	2101, 2102.
SO_2	2411.
Tox-P	2101.
Toxic organics	2331, 2332.
Transportation related	2411, 2421.
Waste oil	2213.